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THE UNIVERSITY OF ALBERTA

BIOPHARMACEUTICAL STUDIES OF SOME PHENTERMINE TABLET FORMULATIONS

by

© Ganga Shankar Pandey

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled, "Biopharmaceutical Studies of Some Phentermine Tablet Formulations", submitted by Ganga Shankar Pandey in partial fulfilment for the requirements for the degree of Doctor of Philosophy in Pharmaceutics.

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This thesis is dedicated to my wife Kusum and our children, Seema, Ratan and Ratna. Their tolerance and understanding during the entire period of this investigation is deeply appreciated.

ABSTRACT

An accurate and sensitive analytical procedure was developed for the determination of phentermine in urine and aqueous solutions utilizing gas-chromatographic techniques.

Four tablet formulations of phentermine hydrochloride were prepared so that their disintegration and dissolution characteristics were different. This was achieved by changing the diluents and the granulating agents in the tablet formulations. The disintegration times and the dissolution rates of the four formulations were determined in three fluids: distilled water, simulated gastric fluid, and simulated intestinal fluid. pH was found to have some influence on both disintegration times and initial dissolution rates. The rotating flask apparatus was found to be suitable for dissolution studies. The U.S.P. disintegration apparatus and the rotating basket apparatus did not give satisfactory results with these formulations.

Urinary excretion profiles following the oral administration of a solution or tablets of phentermine were compared under controlled urinary pH conditions. Both urinary pH and urine volume were found to influence the excretion rate of phentermine in man. A delay in the time of maximum excretion and a lower excretion rate was observed in the case of tablet formulations. Based on the cumulative amount of phentermine in urine, the

bioavailability of each tablet formulation was determined in man in a cross-over study. The urinary excretion method used to evaluate bioavailability of phentermine tablet formulations was found to be satisfactory.

No correlation between the disintegration times and dissolution rate or disintegration times and bioavailability was found for the four phentermine formulations. However, a quantitative correlation between dissolution rates and bioavailability was found. It was concluded that the dissolution rates determined in either of the three dissolution media could be utilized in predicting the bioavailability of these phentermine formulations. This would be useful for quality control purposes.

Some of the pharmacokinetic parameters for phentermine in man were determined by an analog and digital simulation. A one compartmental model seems to adequately describe the pharmacokinetic behaviour of the drug in man.

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INTRODUCTION

The therapeutic efficacy of a solid dosage form can be modified by many factors. It is well known that the absorption characteristics of an orally administered drug product, and consequently its bioavailability can be influenced by the method and materials used in the manufacture of the drug formulation. These formulation effects may result from gross differences in formulation variables, or subtle differences, such as different polymorphic forms of the drug, or minor changes in the inert excipients.

The formulation variables often lead to differences in the dissolution rate of the drug from the solid dosage form. When a drug is administered as a tablet, the active ingredients must dissolve in the gastrointestinal fluid before the absorption can take place. Usually the dissolution process is the slowest step in the dissolution-absorption sequence, and is the rate determining step. Therefore, the dissolution step governs the bioavailability of the drug and in many cases the onset, intensity, and duration of the pharmacological activity of the drug product.

The effects of formulation on the release of drug from a tablet can be studied by measuring the dissolution rate of various formulations. The design and proper selection of the dissolution apparatus is very important in differentiating between different formulations containing the same drug. If a suitable design of apparatus is selected then there is often a good correlation between the <u>in vitro</u>

dissolution rate of the dosage form and its *in vivo* bioavailability.

Also, in any study of a solid dosage form involving the correlation of *in vitro* dissolution data with *in vivo* bioavailability, it is important to select suitable dissolution conditions. In addition to this, the kinetic data obtained for the dissolution of the drug dosage form should be carefully interpreted. Any bioavailability study should be well controlled and the experiments should be designed with sufficient number of subjects. It is important that data obtained in these *in vivo* studies should be analyzed using appropriate pharmacokinetic models in order to obtain a meaningful *in vitro*-*in vivo* correlation.

The following review of the literature represents an attempt to examine the more important factors involved in *in vitro* drug dissolution work and its correlation with *in vivo* bioavailability.

LITERATURE SURVEY

DISSOLUTION STUDIES

When a drug is administered orally as a solid dosage form, one frequently finds that the rate of absorption is controlled by the dissolution process. In other words, the dissolution is the rate-limiting step in the overall process. Thus, in a dissolution rate-limited absorption process, any factor influencing the rate of dissolution must influence the rate of absorption.

Mechanism of Dissolution

Noyes and Whitney (1) quantitatively studied dissolution by rotating cylinders of benzoic acid and lead chloride in water, and analyzing the solution at certain intervals. These workers derived a law which concerns the rate at which solids dissolve in their own solutions when the change in the surface area of exposed solid is negligible. The equation derived by Noyes and Whitney in its original form is as follows:

$$\frac{dc}{dt} = K(C_s - C) \qquad \text{(Equation 1)}$$

where $\frac{dc}{dt}$ is the rate of change of concentration, C_s is the concentration of the saturated solution, C is the concentration at a given time, and K is the proportionality constant. Noyes and Whitney explained the dissolution process on the assumption that a thin layer of the saturated solution was formed at the surface of the solid and the rate at which the solid dissolved was governed by the rate of

diffusion from this saturated layer into the main body of the solution.

In later experiments, Noyes and Whitney (2) and Bruner and Tolloczko (3) incorporated a surface area term (S) into the equation. This modified equation is as follows:

$$\frac{dc}{dt} = K_1 S(C_s - C) \qquad \text{(Equation 2)}$$

Bruner and Tolloczko (3) also showed the dependence of dissolution rate on temperature and rate of stirring.

Subsequently Nernst (4) advanced the concept of the Noyes and Whitney law to include all kinds of heterogeneous reactions and suggested that Equation 1 could be written to include other variables in the following form:

$$\frac{dc}{dt} = \frac{DA}{V\delta}(C_s - C) \qquad \text{(Equation 3)}$$

where D is the diffusion coefficient, V is the volume of the solvent, A is the area of the interface, and δ is the thickness of the stagnant film of the solvent on the solid surface. Brunner (5) also showed that the rate of dissolution depended on the diffusion coefficient. In the theory of Nernst and Brunner, it was postulated that the velocity of the heterogeneous reaction was determined by the velocities of the diffusion process that accompanied it. This included the concept that equilibrium is set up at the boundary surface practically instantaneously compared with the rate of diffusion.

Many workers have published reports which tend to substantiate the film theory of Nernst and Brunner, while other workers have found cases where the theory does not appear to hold. In formulating their theory, Nernst and Brunner assumed that the dissolution process at the surface proceeds much faster than the transport process and that a linear concentration gradient is confined to the layer of solution adhering to the solid surface. Obviously, if the intrinsic reaction rate at the interface was not faster than the rate of the transport process, deviations would occur. Many of the criticisms of the film theory have appeared in the literature (6-13). Wurster and Taylor (14) have discussed these in their review article. However, in general, with some modifications the theory has withstood the test of time.

Hixson and Crowell (15) derived a new law, known as cube root law, in which the velocity of dissolution of a solid in a liquid is expressed as a function of the surface area and concentration. In the derivation of the equations, the authors assumed that dissolution takes place normal to the surface of the dissolving solid, the same effect of agitation is observed on all areas of the solid surface, no stagnation of the liquid takes place in any region and solid particles remain intact throughout the dissolution process.

Hixson and Crowell pointed out that the surface area of a particle was proportional to its weight raised to the

two-thirds power, the proportionality constant being composed of density and volume shape factors. The application of the cube root law has been shown by many workers (16-20).

Danckwerts (21) proposed a model in which macroscopic packets of solvent reach the solid-liquid interface by eddy diffusion in some random fashion. During its residence at the interface a packet is able to absorb solute according to the law of diffusion. Packets at the interface are continuously replaced by new packets of solvent. The surface removal process may then be related to the solute transport rate. Danckwerts applied this model to the dissolution of a gas in a liquid. The rationality of such a model is supported strongly by the work of Fage and Townend (22), who found evidence of turbulent flow in a tube as close as 6 μ from the interface. Johnson and Huang (23) demonstrated the applicability of the model to dissolution from a flat surface into turbulent liquid. Also, Goyan (24), using this model, derived equations for the dissolution of solids in multiparticulate systems.

DISSOLUTION METHODS

Dissolution methods, used both in research and quality control, should meet certain criteria. The most important of these requirements are:

(i) The apparatus must be economically practical and should not be very expensive.

(ii) The apparatus should be scientifically realistic. This requires that the variability within the apparatus should be less than the variability in the products being tested.

(iii) The speed of agitation must be controllable.

Cook (25) has stressed the need for a suitable apparatus in dissolution methodology. Unfortunately, there are as many different types of dissolution rate apparatus as there are investigators studying dissolution. Hersey (26) and Baun and Walker (27) have reviewed several types of apparatus and the variants of these apparatuses. Hersey (26) attempted to classify dissolution methods on the basis of type of agitation and whether the dissolution process occurs under sink or non-sink conditions. It is essential to use an apparatus which operates under sink conditions in order to ensure a good *in vitro*-*in vivo* correlation.

Types of Apparatus

1. The U.S.P.-N.F. Tablet Disintegration Apparatus

This unit is a modified version of the apparatus used by Gershberg and Stoll (28). This apparatus has only one oscillation speed, hence it is not very flexible in use. Primary agitation is produced by the turbulence of fluid flowing through the basket chamber. Because the effective agitation intensity is very high, some poorly available products will tend to release the drug rapidly. Therefore

the use of this apparatus for dissolution studies is not recommended.

2. Rotational Apparatus

This apparatus was first introduced by Souder and Ellenbogen (29). The apparatus consists of a horizontal rotating shaft to which screw-capped bottles are attached. The long axis of the bottle is at a right angle to the axis of the shaft. The rotational speed of 6 - 50 r.p.m. is suggested for this apparatus. To obtain reproducible results, the geometry of the bottles and their positioning relative to the axis of rotation is very critical. For a given speed of rotation, the relative intensity of agitation will depend on the rate of fall of the particles in solution. The rate of fall is related to the density of particles, hence dependent on formulation variables. Other investigators (30,31) have used this apparatus with various sizes of containers. Rosen and Swintosky (32), Rosen (33) and Montgomery and co-workers (34) used radioisotope tracers to study the dissolution of sustained release products.

Hamlin and co-workers (35) have criticized this apparatus. In their study with methylprednisolone polymorphs, significant *in vivo* differences were obtained, but the differences were not detected by the rotating bottle method at 40 r.p.m.

This method is official in the National Formulary XII

(2nd supplement) (36).

Another variant of this apparatus is the Wruble apparatus (37), in which a test tube is attached to a rotating shaft revolving at about 12 r.p.m. This apparatus was used by Hamlin and co-workers (38) to study the dissolution of pellets of methylprednisolone and by Higuchi and co-workers (39) to study micronized prednisolone.

An additional modification of the rotating bottle method was used by Ferrari and Khoury (40). In this method, the drug was dialysed continuously to ensure sink conditions. Gibaldi and Weintraub (41) used the rotating flask assembly to study the dissolution of aspirin formulations at 1.2 r.p.m. and found a good correlation with in vivo data.

3. Rotating Basket Method

This apparatus was first introduced by Searl and Pernarowski (42) and was used to study the dissolution characteristics of a variety of phenylbutazone tablets. Later, Pernarowski and co-workers (43) reported an automated version of the rotating basket method in which a variable speed pump was used to take the test solution through a flow cell in a spectrophotometer.

This method is official in N.F. XIII (44) and U.S.P. XVIII (45). A rotational speed of 50 r.p.m. is reported to be a realistic rotational speed, but at this speed homogenity of bath fluid may present a problem. A major objection to this apparatus appears to be clogging of the basket

by some dosage forms. This could be a serious defect of this apparatus. Another study (46) showed the variations in results due to vibrations produced by the motor in the rotating basket assembly. Rosolia and co-workers (47) have shown that variation in dissolution results from the use of different shaped vessels.

4. Beaker Method

This method was first used by Levy and Hayes (48). This method is one of the simplest, yet most widely used. It has been successfully used by Levy and co-workers in a variety of situations (49-54).

Variations in dissolution were found to depend on the location of tablets in the bottom of the beaker. To overcome this problem, Flanagan and co-workers (55) used a rectangular basket to center the tablets on the bottom of the beaker.

Niebergall and Goyan (17) developed an automatic recording device for use with the beaker method. Another automated method operating under sink conditions has been described by Richter and co-workers (56). Gibaldi and Feldman (57) used a layer of an organic solvent over the aqueous dissolution medium to ensure sink conditions. In yet another modification, a capsule was attached to the base of the stirrer as reported by Paikoff and Drumm (58). A multiple station apparatus capable of sampling automatically and simultaneously at predetermined time intervals has been

described by Castello and co-workers (59).

5. Stationary Basket Method

The stationary basket method was developed by Cook and co-workers (60). In this method the dosage form is put in a stationary basket and agitation is produced by a stirrer.

The stationary basket method was used by Middleton and co-workers (61) to study the dissolution of formulations of para-aminosalicylic acid.

6. Dialysis Method

The dialysis method has been used by many investigators to achieve sink conditions. Marlowe and Shangraw (62) reported the use of a dialysis cell similar to that reported by Patel and Foss (63). In this method, a tablet is placed on one side of the dialysis membrane, and the cell is rotated at 15 r.p.m. The method is discontinuous since the operation has to be stopped to withdraw samples. This difficulty was overcome by the automated dialysis apparatus of Barzilay and Hersey (64).

In the dialysis method the data collected are a resultant of not only the disintegration, deaggregation and dissolution processes but also of membrane transport. Introduction of dialyzing membrane into dissolution rate apparatus seems to needlessly complicate an already complicated situation.

7. Miscellaneous Methods

There are many modifications of the methods discussed which may fall into this category. The review articles (26, 27) give an excellent account of these methods. Some of the more commonly used methods are briefly discussed here.

Tape Method

In the tape method, developed by Goldberg and co-workers (65), the particles are dusted on a pressure sensitive tape and the whole assembly is inserted into the solution. The dissolution of the drug into the stirred solution is monitored. The method has been successfully used by several authors (19,20,65-67).

Direct Particle Size Measurement

The Coulter Counter has been used to follow directly the dissolution rate of drugs (68). Many reports on the use of the Coulter Counter to study the dissolution rate of particles have been reported (69-73).

Automatic Methods

Automatic dissolution methods involve a circulation of the dissolution medium through a dissolution cell, a filter and suitable measuring device. The circulating fluid generally provides the necessary agitation. The first flow-through dissolution cell was reported by Myers (74). The apparatus consisted of a stoppered cylindrical tube with a filter above the bottom outlet and an inlet for returning the fluid to the reservoir. A pump was used to circulate

the fluid. A modification of this apparatus was reported by Baun and Walker (27).

Niebergall and Goyan (17) developed an automatic recording apparatus for use with the beaker method of Levy and Hayes (48). Richter and co-workers (56) described an automatic beaker method operating under sink conditions.

An automatic dissolution apparatus based on the official disintegration apparatus has been described by Schroeter and Wagner (75) and Schroeter and Hamlin (76).

A continuous flow apparatus for dissolution rate study of prednisolone tablets and powder has been reported by Tingstad and Riegelman (77). Ullah and Cadwallader (78) developed a three compartmental system for dissolution studies of slightly soluble powders under sink conditions. The apparatus was designed to produce sink conditions by the use of an organic phase in which the drug was very soluble. A barrier in the dissolution medium was also included in the apparatus, which prevented floating powders from entering and dissolving directly in the sink phase.

Vertical column apparatuses have been used by many workers to study the dissolution of solid dosage forms (79-81).

Dissolution Studies with Tablets

It is very important to realise the need for selection of a suitable dissolution rate apparatus for studying the

dissolution characteristics of solid dosage forms. Almost every available dissolution method has been used by one worker or another. However, the most important point is to specify the full description of the apparatus used, along with the complete experimental conditions. The in vitro test conditions giving results which correlate well with the in vivo results may be different for different drugs and even for different tablets containing the same drug. It is possible to establish conditions for an in vitro dissolution rate test for each drug which will give results correlating well with in vivo results for a wide variety of tablets and capsules containing the same drug. The test conditions established for even a large number of drugs cannot be just blindly applied to a new drug and its dosage forms.

Huber and co-workers (82), Vliet (83), Cooper (84), Kaplan (85), Schroeter and Hamlin (76), Schroeter and Wagner (75), and Lazarus and co-workers (86) have used modifications of the U.S.P. disintegration apparatus for dissolution studies from tablets and capsules.

The beaker method of Levy and Hayes (48) has been used by many investigators. A stirring rate of 30-60 r.p.m. was generally used (87). There was sufficient agitation to obtain a homogeneous solution for sampling purposes yet low enough not to break down the 'microenvironment' of the tablet

being tested (49). Stirring apparatuses using different stirrers, stirring rates, and containers for dissolution fluid and test preparations were used by Gibaldi and Feldman (57) and by Wood and Syarto (88). Shepherd and co-workers (89) used a magnetic basket assembly, which was a modified version of the beaker method to study the dissolution profiles of capsules and tablets.

The rotating bottle method, which is official in the National Formulary XII, 2nd supplement (36) has been used to study dissolution rates by many workers (90-93). Bottles of various sizes and fluid volumes from 60 - 100 ml were used. Rotation speeds of 12 - 44 r.p.m. were used in most studies. Recently Simmons and co-workers (94) reported a rotating compartmentalized disk for dissolution rate determinations for capsules and tablets.

All the apparatuses used have a wide range of agitation intensity under which tests may be conducted. The need for low agitation intensities in disintegration studies has been stressed by Filleborn (95). Similar views were advanced for dissolution studies by other workers (35,96).

Interpretation of Dissolution Rate Data

Wagner (97) has offered a good review of the interpretation of dissolution rate data from tablets and capsules. It has been shown by many authors that the dissolution process obeys first order kinetics. Gibaldi and Feldman (57) derived an equation to describe the dissolution process

under sink conditions as a first order rate process. The equation derived is as follows:

$$\log A = \log A^{\infty} - \frac{K}{2.303} t \qquad \text{(Equation 4)}$$

where A is the amount of drug undissolved at time t, A^{∞} is the amount of drug in solution at 'infinite' time, and K is the rate constant for the dissolution process. In the derivation of the above equation, it was assumed that the surface area was proportional to the weight of undissolved drug. A similar equation was derived by Wagner (97). Accordingly, percent drug not dissolved is plotted on the logarithmic scale of semilogarithmic graph paper against time. Frequently, the data points are nonlinear in the early time period, but at later times a straight line is obtained. Such first-order plots are obtained under sink conditions, as pointed out by Gibaldi and Feldman (57).

Wagner (97) introduced the surface area concept to interpret dissolution rate data. Under sink conditions a percent dissolved value at any time may be regarded as the percent surface area generated at that time. Using the Noyes and Whitney equation (2), Wagner (97) derived the following equation:

$$\text{\% dissolved at time t} = \frac{W}{W^{\infty}} \times 100$$

$$= \frac{\int_0^T S(t)dt}{\int_0^{\infty} S(t)dt} \times 100 \qquad \text{(Equation 5)}$$

= surface area generated to time T of total surface area generated

where W is the cumulative amount dissolved to time T, and W^{∞} represents the amount in solution at 'infinite' time.

If Equation 5 is valid, then percent dissolved-time data derived from <u>in vitro</u> testing of tablets and capsules may best be described by a distribution function, such as the logarithmic-normal or logarithmic-logistic distribution functions. If the dissolution data follows a logarithmic-normal distribution, then one expects a single linear line when percent dissolved values are plotted on the probability scale against the time values on logarithmic scale of log-normal paper.

It has been shown by Gibaldi and Feldman (57) and Raghunathan and Becker (98) that the release of drug in a quantity of solvent just sufficient to dissolve the total amount of drug in the dosage form may obey apparent second-order kinetics. Therefore the importance of establishing sink conditions is essential in dissolution studies.

An exponential release of aspirin from various matrices in tablet form has been reported by Wiseman and Federici (99). The authors derived the following equation

to explain the data:

$$R = R_0 \cdot e^{K(t-t_0)} \qquad \text{(Equation 6)}$$

where R is the percent released, R_0 is the amount released at time t_0 when exponential release began, K is the rate constant, and t is the time. A linear plot of percent aspirin released on the logarithmic scale versus time was obtained.

Higuchi (100) derived equations for the situation in which a drug is dispersed as a solid in a matrix. In the case of diffusion-controlled release of the drug from the matrix, a plot of drug released per unit surface area versus square root of time gives a linear relationship. Desai and co-workers (101-104) have shown that such linear square root versus time plots were obtained with solids dispersed in matrices.

BIOAVAILABILITY

The bioavailability of a drug dosage form can be defined as the amount of drug which may be obtained from a given dosage form under specified conditions. Bioavailability is usually measured relative to a 'standard preparation' which is usually given a value of 100% bioavailability (105). This criterion is very useful in deciding whether one formulation of a drug is better than the other formulation and thus answers many questions during dosage form design.

There are several methods available for estimating bioavailability. One could measure the area under the blood concentration curve or measure the amount of unchanged drug in urine for at least six or seven biological half-lives. These methods have been reviewed by Wagner and Nelson (106) and Wagner (107).

As pointed out by Wagner and Nelson (106), it is important to collect urine for a long period of time so that essentially all drug excreted in the urine is collected. Otherwise the estimated bioavailability will be incorrect. To compare the bioavailability of two or more products, the following relationship may be used:

$$A_u^\infty = f \cdot F \cdot D \qquad \text{(Equation 7)}$$

where A_u^∞ is the amount of unchanged drug excreted in urine in 'infinite' time, f is the fraction of drug reaching the circulation which is excreted unchanged, F is the fraction of dose absorbed, and D is the dose of the drug. Hence, if one administers the same dose of drug in two or more dosage forms, then D is held constant, and the ratio of unchanged drug in urine excreted will give the relative bioavailability. It should be noted that this involves the assumption that f remains constant in a given individual. Hence one would expect crossover studies to be most reliable.

There are many formulation factors which influence the bioavailability of a product. These factors could arise

from various sources during the formulation procedure. Barr (108) has listed the factors involved in the assessment of availability of drug products. These are: excipients used in solid dosage forms, particle size, disintegration time, and dissolution rate of the finished product, polymorphism. Many examples of poor bioavailability due to formulation effects have appeared in the literature. Different commercial brands of chloramphenicol have been studied by Clazko and co-workers (109). The differences in availability of the drug were found to be due to differences in particle size of the drug and the use of different polymorphic forms. Juncher and Raaschou (110) have reported the differences in bioavailability of different commercial products of penicillin V arising from differences in salt forms of penicillin V. Engle (111) studied four brands of phenindione tablets and reported that differences in bioavailability were due to the variation in particle size. Many authors (112-114) have reported the lack of hypoglycemic effect of tolbutamide formulations arising from differences in binders used and other formulation variables. Differences in plasma levels in commercial brands of griseofulvin have also been found to be due to differences in particle size (115,116). The plasma level was found to correlate well with the surface area of the particles of griseofulvin. Some reports (117-120) on the bioavailability of riboflavin from different brands of sugar-coated

multivitamin tablets have appeared. The large differences were attributed to formulation factors, particularly to the coating procedures. Morrison and Campbell (119) and Levy (49) studied the bioavailability of different formulations of aspirin tablets. In these studies large differences in the bioavailability have been found. Hollister and Kanter (121) compared the bioavailability of uncoated and enteric-coated aspirin tablets. The bioavailabilities were found to be similar in both cases. Pfeiffer and co-workers (122) also studied the bioavailability of aspirin tablets in man. These authors have used the electroencephalogram (EEG) to detect the differences in bioavailability. The authors claimed that careful pharmaceutical formulation produced a greater 'aspirin effect' on the human brain as indicated by the EEG. Martin and co-workers (123) reported that the formulation factors affected the bioavailability of sulfisoxazole tablets. In their investigation urinary excretion studies were conducted. The authors reported that the rate of urinary excretion of the drug was significantly different with different tablet formulations; however, the extent of urinary excretion remained the same.

Some authors (123, 124) reported large differences in the bioavailability of sodium diphenylhydantoin capsules. Large differences in bioavailability of tetracycline formulations have been reported by Barr (108) and Macdonald and co-workers (125).

Chapman and co-workers (126) studied the bioavailability of different brands of sugar-coated tablets of para-aminosalicylic acid. Large differences in bioavailabilities were found between the brands studied. Middleton and co-workers (61) found the significant differences in bioavailability between the compressed and enteric-coated tablets of p-aminosalicylic acid and its sodium and calcium salts.

The bioavailability of oxytetracycline hydrochloride capsules from 13 different manufacturers were compared in a study by Brice and Hammer (127). Terramycin® was found to be superior to all other products as indicated by average serum levels in crossover study. The investigation of Blair and co-workers (128) gave results similar to those obtained by Brice and Hammer (127).

IN VITRO - IN VIVO CORRELATIONS

There are two types of correlations used to compare *in vitro* and *in vivo* results depending on whether the data are parametric or non-parametric in nature. The parametric method of correlating *in vitro* and *in vivo* data is strictly a quantitative statistical method. On the other hand, the non-parametic method is applicable to discrete data treated qualitatively on the basis of class intervals. Both parametric and non-parametric data could be analyzed by the rank order correlation method. Wilcoxon's Signed Rank Test (129)

may be employed for non-parametric data.

Quantitative Correlation

In quantitative correlation the in vivo variable (y) is related to the in vitro variable (x) by an equation. The equation may take various forms, such as:

$$y = b \cdot x \qquad \text{(Equation 8)}$$

$$y = a + bx \qquad \text{(Equation 9)}$$

$$\log y = \log y^{0} - bx \qquad \text{(Equation 10)}$$

where b is the slope of the line and a is the intercept of the line on the y axis. It is important to realize that such correlations should be tried only when there is a good theoretical basis. Also, the data should be of good quality and should be sound theoretically. To test the significance of such a correlation, Pearson's product moment correlation (130) has been most widely used. One calculates the values of the correlation coefficient (r) and compares its value to table values of r to obtain the level of significance. For predictive purposes, the coefficient of determination (r^2) is calculated. The quantity $100r^2$ is the percentage of variance of the y values which may be accounted for by the difference in the x values. It is suggested (131) that the $100r^2$ value should be very large (between 90 and 100) for good correlation. If the $100r^2$ value is much smaller than 100 then the confidence limits of a predicted y value

will be large for a given x value. Due to wide variability in *in vivo* work, it is often extremely difficult to obtain a highly significant correlation.

Rank Order Correlation

In rank order correlation the *in vivo* variable (y) either (i) increases as the *in vitro* variable (x) increases, or (ii) y increases as x decreases, or (iii) y decreases as x increases. Here the variables are assigned a rank and then the ranks are treated statistically.

Wagner (131) has discussed in detail the variables which have been derived from *in vitro* and *in vivo* experiments and suggested which variables should be correlated. From Wagner's discussion it seems that the time for 50 percent of the drug to dissolve *in vitro* ($t_{50\%}$) is probably the best *in vitro* variable to correlate, since its value indicates the central tendency of the dissolution data, and by use of this value one has not committed oneself to any formal kinetic interpretation of the data. If absorption of the drug is dissolution rate controlled *in vivo*, then the half absorption time obtained by blood or urine data is probably the best variable to estimate and correlate with $t_{50\%}$ obtained from dissolution data. There are several examples of rank order and quantitative correlations which have been reported in the literature.

Levy and co-workers (132) studied the three brands of acetylsalicylic acid tablets. The amount of salicylate

excreted in urine in 12 hours was found to have rank order correlation with the amount dissolved in 10 minutes in 0.1N hydrochloric acid. In this study, the beaker method of Levy and Hayes (48) was used for dissolution studies. In another study, Levy and Sahli (133), using the urinary excretion study in man, studied the bioavailability of aspirin and aluminum acetylsalicylate. A 100 mesh powder was used for in vivo studies. The in vitro studies were performed with the drug in the form of pellets mounted in plexiglass holders. The dissolution rate was determined in 0.1N hydrochloric acid in a three-necked round bottom flask using a rotational speed of 555 r.p.m. A good rank order correlation was observed between the amount of salicylate excreted in urine and the in vitro rate of dissolution. Levy (49) reported a quantitative correlation between the mean amount of salicylate excreted in the urine in one hour after administration of aspirin and the amount of aspirin dissolved. Administration of water following the drug administration caused higher amounts of salicylate to appear in the urine. However, in both cases a quantitative correlation was obtained. Wood (134) reported a quantitative correlation between the serum salicylate concentrations in man and percentage of drug dissolved in vitro in a given time. Levy and Hollister (54) introduced a new approach to quantitative correlation, and this method was further elaborated for aspirin formulations by Levy and co-workers (135)

and Levy (136). In this method an intensity factor (I) is determined. To correlate the *in vivo* with *in vitro* data, the percent of dose absorbed to each time T is plotted against the percent dissolved *in vitro* at time (T - lag time)/I. This type of plot yielded a straight line.

Gibaldi and Weintraube (41), using the rotating flask method for dissolution studies, reported a quantitative correlation of several dosage forms of aspirin. A rotational speed of 1.2 r.p.m. was used and 0.1N hydrochloric acid was used as the dissolution media.

Katchen and Symchowicz (137) reported the plasma concentrations of griseofulvin after a single oral dose in human subjects. A good quantitative correlation was observed between mean plasma concentration and logarithm of amount of drug dissolved in 30 minutes. In another report, Symchowicz and Katchen (138) found a rank order correlation between griseofulvin plasma concentrations and the logarithm of drug dissolved in 30 minutes in the *in vitro* test. Similar results were obtained following the multiple dose of griseofulvin in man and dog studies. Simulated intestinal fluid was used as the dissolution medium in both the studies (137, 138).

In a study by Wagner (139) using tolbutamide tablet formulations, the blood sugar lowering effect of the drug was studied in man. A quantitative correlation was observed between the blood sugar level and the logarithm of the time

for 20 percent of the tolbutamide to dissolve in an *in vitro* test. In another study with compressed disks of tolbutamide and three of its salts, Nelson and co-workers (140) studied the urinary excretion of its metabolites, blood lowering effects of the same disks and the *in vitro* rate of dissolution. The urinary excretion, hypoglycemic effect and *in vitro* rates of dissolution rank-order correlate for the sodium salt, 2-amino-2-methyl-1-propanol salt and tolbutamide free acid. Results for the 1-amino-2-propanol salt of tolbutamide did not correlate as well.

Rank-order correlation between the average amount of tetracycline and its salts excreted in urine and *in vitro* dissolution rate in neutral and alkaline intestinal fluid was reported by Nelson (141). The drugs were administered to human subjects in the form of pellets packed in capsules with sodium bicarbonate.

A clinical study was conducted by Campagna and co-workers (142) using prednisone tablets meeting U.S.P. XVI specifications and another brand of prednisone which was found to be clinically ineffective. It was found that both the formulations passed the U.S.P. XVI disintegration test. However, the dissolution tests indicated that there was a rank order correlation between the clinical results and the time for 50 percent of the drug to dissolve *in vitro*. When the disintegration test was repeated without the use of disks in the apparatus, a better rank order correlation

was found.

Hamlin and co-workers (35) and Ballard and Nelson (143) reported the in vivo and in vitro studies of two polymorphic forms of methylprednisolone (form I or II). Pellets of each form were implanted in rats. After different time intervals, the pellets were removed and the rate of dissolution was determined from the loss of weight of the pellets. This dissolution rate was found to have rank order correlation with the in vitro dissolution rate determined by the method of Nelson (144).

In vitro-in vivo correlation in man for three formulations of sulfamethazine tablets was reported by Taraszka and Delor (145). The comparison was made between the average areas under the total plasma concentration curve in man and the times for 50 percent of the drug to dissolve in vitro. A good rank order correlation was observed.

Bates and co-workers (146) reported a rank order correlation between the rate of dissolution and absorption of salicylamide from tablet and suspension dosage forms. The cumulative amount of the total salicylamide excreted in urine was determined. The in vitro data were obtained by the flask-stirrer method at 70 r.p.m. using 0.1N hydrochloric acid as the dissolution medium. Using the data from the same study, Bates and co-workers (146) showed a quantitative correlation between cumulative percent of the dose of salicylamide excreted in the urine in one hour and

percent salicylamide in solution after 15 and 30 minutes.

The anhydrous and trihydrate forms of ampicillin were studied *in vitro* and *in vivo* by Poole and co-workers (147). Capsules and suspensions of both the forms were administered to dogs and man in cross-over fashion. The area under the serum concentration curve was correlated with *in vitro* data and a rank order correlation was observed.

Shenoy and co-workers (30) showed a quantitative correlation of percent amphetamine released *in vitro* in one hour with *in vivo* availability in man for eight different brands of sustained release capsules of amphetamine. Urinary excretion studies were performed to get *in vivo* data. Beckett and Tucker (148, 149) reported the urinary excretion studies on various dosage forms of amphetamine and related compounds in humans under controlled urinary pH conditions. The authors attempted to correlate the *in vivo* data with *in vitro* dissolution of the drugs. In a similar study Jun and co-workers (150) showed a good *in vitro*-*in vivo* correlation for a prolonged-release preparation of chlorphentermine. These workers obtained the *in vivo* data under controlled urinary pH conditions.

ROLE OF PHARMACOKINETICS

The use of pharmacokinetic methods for the treatment and interpretation of *in vivo* data is increasing because its use gives a better understanding of drug absorption,

distribution, and elimination processes. Using these methods, the determination of the rate constants of absorption, distribution and excretion along with other biopharmaceutical and pharmacokinetic parameters becomes easy. complexity of the biological system makes the above-mentioned objectives difficult to achieve, but the development of compartmental models has made the task simpler.

The concept of a compartmental model was first introduced by Teorell (151), and later mathematically developed by Solomon (152) and Bellman and Roth (153). These models assumed that the body can be described as one or more compartments connected to each other in which the amount of unchanged drug or its metabolite is uniformly distributed. The transfer of drug or its metabolite from one compartment to another by a reversible or irreversible process is usually adequately described by first order kinetics. This finding has definitely facilitated the mathematical development of compartmental models and their application to the biological system.

Wagner (154, 155) has discussed in detail the suitability of mathematical models in pharmacokinetics. Berman and Schoenfeld (156) have pointed out the problems associated with the use of compartmental analysis. These authors stated that the compartment is really an 'average' rather than an 'exact' description of the biological system. Similar views were expressed by Nooney (157).

The data derived from the experiments involving drug absorption, distribution, metabolism, and excretion consist of measurement of unchanged drug or its metabolite in serum, plasma, blood, cerebrospinal fluid, and other body fluids. Similar measurements may also be made on urine. After fitting the experimental data to the mathematical equations, one can ascertain the suitability of the model on the basis of the agreement between the theoretical and experimental values.

The first kinetic study of drug absorption and elimination was performed by Widmark (158, 159) using acetone. He recognized that the drug was both metabolized and excreted in the urine as well as eliminated via the lungs. He found that after absorption of the dose, the concentration of acetone in blood decreased exponentially and the rate of excretion was directly proportional to the concentration in the blood. Later Dominguez (160-162) and Dominguez and Pomerence (163, 164) pointed out the relationship between the plasma concentration and the excretion rate and significance of the parameters involved in the distribution and excretion of drugs.

Based on the findings of Widmark, Dominguez and Pomerence (158-164), the kinetics of drug absorption, metabolism, distribution, and excretion have undergone tremendous expansion. A thorough mathematical treatment has been given since then, which has been reviewed and presented in

various papers (165-167).

Though most of the kinetic studies performed were treated as one compartmental models, the application of two compartmental models has been discussed by several authors (168-170). Recently, Riegelman and co-workers (171, 172) and Loo and Riegelman (173) demonstrated the suitability of a two compartmental model to depict the distribution of drugs in the body. They proposed several equations to calculate the various pharmacokinetic parameters using a two compartmental model. Several reports on two compartmental systems have appeared in the literature in recent years. Kaplan (174) studied the pharmacokinetic profile of coumermycine A_1 following intravenous and oral administration. Rowland and co-workers (175, 176) applied the two compartmental model to study the absorption, distribution, metabolism, and excretion of acetylsalicylic acid. Runkel and co-workers (177) did a similar study with naproxen in various laboratory animals and human subjects.

One should be very cautious in using the two or multicompartmental models in predicting the pharmacokinetic parameters. There are several problems associated with the use of the pharmacokinetic models in general and two or multicompartmental models in particular. Westlake (178) has pointed out some of the problems associated with such studies. He suggested that the pharmacokinetic parameters obtained by fitting blood levels of a drug may be in error.

These errors are probably unimportant as long as the model is used to predict the blood levels, but use of these parameters to predict other features of the system, e.g. tissue drug levels, may lead to considerable error. Similarly in other reports, Wagner and Metzler (179) and Wagner (180) have shown that it is possible to predict blood levels after multiple doses from the single dose level data, and also it is possible to fit the data to either one or two compartmental models. The authors suggested that one may expect only a small error in predicting multiple dose blood levels compared with other possible sources of error. In view of these findings it may be suggested that the chosen pharmacokinetic model should be as simple as experimental results permit.

The determination of absorption rate constant and subsequently construction of percent absorbed-time plots from the blood or urine data is well established (181, 182). However, these methods have largely been supplanted by the simplified approach of Wagner and Nelson (106, 183). The method of Wagner and Nelson allows the calculation of apparent volume of distribution or the fraction of drug excreted unchanged.

The equation developed by Nelson and Schaldemose (182) to calculate the absorption rate constant from the urinary excretion data requires the measurement of unchanged drug in urine at various time intervals. Some possible

errors in applying these methods are discussed by Wagner (184).

COMPUTERS IN BIOPHARMACEUTICS

In biopharmaceutics and pharmacokinetics, analog and digital computers are used extensively to evaluate the rate constants of drug transfer across various body membranes. The rate constants obtained by the computer methods are more reliable and accurate compared to more conventional methods such as classical mathematical analysis or graphical methods.

The fundamentals and philosophy of the use of electronic analog computers in biopharmaceutics have been discussed by Garrett and co-workers (185). Although analog computers are less accurate compared to the digital computers, they are simpler and more flexible, particularly in solving differential equations. Analog computers are convenient to use because it is possible to visually compare the computer generated curves with the experimental data. Their use has been successfully extended into biopharmaceutics permitting one to confirm the validity of a chosen compartmental model and to obtain the various rate constants of absorption, distribution, metabolism, and excretion. Determinations of rate constants are carried out by describing the various processes with a set of differential equations, and then appropriately programming the analog

computer. The results are normally obtained as plotted curves of drug amounts versus time for any selected compartment of the model. In analog computing, two programming methods are utilized. In one, the dependent variables is plotted as a continuous function of time. The computer is usually programmed on the basis of a specific equation as shown by Taylor and Wiegand (186). More commonly the curve fitting procedure as suggested by Garrett and co-workers (187) is used.

Garrett and co-workers (185) studied the mechanism and rate of gastrointestinal absorption, tissue distribution, and elimination of psicofuranine. This method was used to study multiple dose kinetics by Wagner and Alway (188). There are reports in the literature indicating the usefulness and versatility of analog computers in pharmacokinetic studies (189-191). In addition to this, the behavior of prolonged-action preparations have been studied using analog computers (192-194). In vivo release from drug products has been simulated from blood data using the analog computer (195, 196). Rowland and co-workers (176) used an analog computer to study the absorption kinetics of aspirin in man following its oral administration. Garrett and Lambert (197) described the techniques of using an analog computer in the preparation of drug formulations with improved therapeutic efficacy. In this study five basic dosage forms were used to show the versatility of analog

computers and the variety of programs that are possible.

Beckett and Tucker (149) and Beckett and co-workers (198-200) studied the kinetics of a number of amphetamine-like compounds. These studies included the examination of the quantitative relationship between urinary pH and kidney reabsorption of drugs (198), pharmacokinetic and biopharmaceutical studies with amphetamine-like drugs (149), kinetics of buccal absorption of drugs (199), and prediction of the distribution and excretion of drugs under conditions of fluctuating urinary pH (200). Jun and co-workers (150) studied the pharmacokinetics of chlorphentermine in man under acidic urine pH conditions, and assuming a two compartmental model, fitted the urinary excretion data using an analog computer.

Nonlinear least square estimation of the rate constants using high speed digital computers has also been used quite frequently. The results are more reliable than conventional graphical methods. The preliminary estimates of the rate constants obtained graphically are used as input to an iterative digital computer program. A computer program NONLIN (201) has been used by many workers to estimate the rate constants in a variety of situations (115, 202-204). Smolen (205, 206) has used another but similar program in the study of mydriatic drugs. Saunders and Natunen (207) have developed a digital computer program for various pharmacokinetic calculations.

As presented, the review of the literature reveals that the interpretation of dissolution and bioavailability data and their correlation is essential for the development of a therapeutically effective solid dosage form. Further, little or no research work has been reported in the literature regarding development of phentermine tablet formulations. Therefore, the need for further investigation in this area has led to the present investigation.

AIMS AND OBJECTIVES OF THE PRESENT INVESTIGATION

The aims of the present investigation were:

1. To formulate four tablet formulations of phentermine hydrochloride having different dissolution characteristics without changing too many formulation variables.

2. To study the dissolution profile of these formulations using three different dissolution apparatuses which differed in the intensity of agitation and mixing of the dissolution media.

3. To study the bioavailability of these formulations in man, evaluated by measuring the amount of unchanged drug in urine under acidic urine pH conditions.

4. To correlate the *in vitro* and *in vivo* results.

5. To evaluate some of the pharmacokinetic parameters of phentermine using analog and digital computers.

EXPERIMENTAL

FORMULATION OF THE TABLETS

Four tablet formulations containing 12.5 mg phentermine hydrochloride (equivalent to 10 mg phentermine base) were prepared. The formulation factors were controlled so that each batch of tablets had different disintegration and dissolution characteristics.

A batch of 500 tablets, each weighing 250 mg, was prepared according to the general formula in Table I.

Table I

General Formula for Phentermine Tablet Formulations

Ingredients	Quantity for one tablet	Quantity for 500 tablets
Phentermine hydrochloride	12.5 mg	6.25 g
Diluent	200.0 mg	100.0 g
Granulating agent	q.s.	q.s.
Disintegrating agent	25.0 mg	12.5 g
Lubricant	2.5 mg	1.25 g

The drug, diluent, and half of the corn starch used as the disintegrating agent were mixed thoroughly in a wedgwood mortar, and the mixed powders were passed through a No. 40 sieve. A 10% w/w suspension of starch in water was then prepared. The suspension was placed in a beaker and heated on a water bath and the contents were stirred until a smooth paste was obtained. Sufficient starch paste was added to the mixed powder in the mortar and the contents

were thoroughly mixed until a mass of suitable consistency was obtained. The weight of the granulating fluid was recorded. The granules were obtained by the wet granulation process by forcing the wet mass through a No. 8 sieve. The granules were then evenly spread on a tray and dried in an oven at 50 degrees for one hour. The granules were then passed through a No. 14 sieve to break the lumps. The weight of the dry granules was determined at this point. The remainder of the starch powder as the disintegrating agent was mixed with the granules. A 20% solution of light mineral oil in chloroform was sprayed on the granules using an aerosol spray. The granules were then spread evenly on a piece of paper and were left at room temperature for one hour to remove the solvent. The tablets were made on a single punch, power driven tablet machine[a] using a 3/8 inch standard concave die and punch assembly. The tablet hardness was adjusted to a reading of 5 on the Strong Cobb tablet hardness tester[b].

In preliminary testing, capping of tablets was found to be a problem with all the formulations. This was thought to be due to the very "fluffy" nature of the phentermine hydrochloride powder. To overcome this problem, the drug was dissolved in a predetermined quantity of the granulating fluid. In this manner, the drug was thoroughly wetted prior

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[a] Manesty Machines Ltd., Liverpool, England, Model F-3

[b] Strong Cobb Arner Inc., Cleveland, Ohio

to the granulation process, and satisfactory tablets were then obtained. The formulation variables of each formulation are shown in Table II.

Table II

Formulation Variables of Phentermine Tablets

Formulation No.	Diluent	Granulating Agent
I	lactose	starch paste (10%)
II	calcium phosphate	starch paste (10%)
III	dextrose	starch paste (10%)
IV	dextrose	starch paste (5%) and acacia (10%)

WEIGHT VARIATION TEST

A weight variation test on each tablet formulation was performed according to the U.S.P. (208). Twenty tablets were weighed and the average weight was calculated. Then each of the 20 tablets was individually weighed and the percent deviation was calculated.

CONTENT UNIFORMITY TEST

In order to determine the uniformity of phentermine content in each tablet formulation, one tablet was dissolved in distilled water and volume was made up to 100 ml in a

volumetric flask. From this an aliquot of 0.5 ml was withdrawn and was analyzed for the phentermine content using GLC technique. Ten tablets from each formulation were analyzed.

DISINTEGRATION TEST

The U.S.P. disintegration test (45) was carried out to determine the disintegration times of each tablet formulation. A U.S.P. disintegration apparatus[a] was used which consisted of a basket rack assembly, a one liter beaker, and a thermostatically controlled water bath. Nine hundred milliliters of disintegration fluid was used in each case. One tablet was placed in each of the six tubes and the time required to disintegrate all the tablets was recorded. The disintegration test was carried out using distilled water, simulated gastric fluid (209) and simulated intestinal fluid (210). The temperature of the disintegration fluids was maintained at 37°.

URINARY EXCRETION STUDIES

The urinary excretion studies have been used extensively to study the bioavailability of drug products. The method is very convenient and for this reason it was employed in the present investigation. Also it is known in the case of many basic drugs that the urinary excretion rate

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[a] Van-Kel Industries, Livinston, New Jersey

is dependent on the pH of the urine, and to some extent, on the urine flow rate (150). Therefore, to achieve the maximum recovery of the drug from the urine, the urinary excretion studies were performed under controlled acidic urine pH conditions.

Analytical Procedure

Extraction of the Drug from Urine

The extraction procedure was similar to that previously reported for phentermine (211) and chlorphentermine (150). A 5 ml urine sample was pipetted into a 15 ml glass centrifuge tube containing 1 ml of a 50 mcg ml^{-1} solution of chlorphentermine as an internal standard. To this was added 0.5 ml of 20% w/v sodium hydroxide solution and then 2.5 ml 'Reagent' grade diethyl ether. The tube was tightly stoppered and shaken for five minutes, followed by centrifugation at 2000 r.p.m. for 10 minutes. The upper ethereal layer was transferred to a glass sedimentation tube. Two more extractions were performed. The combined ethereal extracts were reduced to a small volume (about 50 µl) in the sedimentation tube using a water bath at 40°. A small boiling stone was added to avoid bumping of the solution. Then one to five µl of the concentrate was injected onto the gas-liquid chromatographic column.

Gas-liquid Chromatographic Procedure

A Perkin-Elmer 990 gas chromatograph[a] equipped with dual flame ionization detectors and a 0 - 10 mv Speedomax W recorder[b] was used. A chart speed of 0.5 inch min^{-1} was employed throughout the experimental work. All samples were injected with a 10 µl syringe.

The chromatographic column was a 1/4 inch O.d. coiled glass column, six feet in length and packed with 3% OV-17[c] on Chrom G-AW-DMCS (H.P.)[d] having a mesh size of 60-80. The column was silanized with 2 X 6 µl injections of hexamethyldisilizane, and equilibrated for 24 hours at the operating conditions.

The oven temperature used was 150°. The injection port temperature was maintained at 220° and manifold at 200°. Helium was used as the carrier gas at the rate of 42 ml min^{-1} with an inlet pressure of 28 psig. Air and hydrogen inlet pressures were 25 and 30 psig respectively. The optimum air and hydrogen pressures were selected for the maximum response of phentermine and the internal standard by measuring the peak areas at different gas pressures.

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[a] Perkin-Elmer, Norwalk, Connecticut

[b] Leeds and Northrup Co. Inc., Philadelphia, Pennsylvania

[c] Dimethylsilicone Gum

[d] Diatomaceous Silica aggregate

The peak areas were measured using a planimeter[a].

Analysis in Urine

The analysis of 'blank' samples of urine collected from each subject was performed for every excretion trial. The absolute recovery of phentermine extracted from urine was determined by comparing the peak area ratios of known amounts of the drug dissolved in ether to that obtained from an equal amount of the drug dissolved in blank urine.

The reproducibility of the extraction procedure was confirmed by extracting 12 urine samples each containing 10 mcg ml^{-1} phentermine hydrochloride and analysing for phentermine content.

In order to investigate any possible deterioration of phentermine in urine, a urine sample containing the drug was refrigerated at 4° for two weeks and analysed at the selected time intervals.

A calibration curve was prepared from stock urine solutions containing known amounts of phentermine hydrochloride. Urine samples containing 2.5 - 60 mcg of phentermine were assayed by the described analytical technique. The peak areas of phentermine and chlorphentermine were measured with a planimeter. A calibration curve was then constructed by plotting the ratio of peak area of phentermine to chlorphentermine (internal standard) against known

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[a] Gelman Instrument Company, U.S.A.

amounts of phentermine in urine. The calibration procedure was repeated at one to two month intervals.

The use of the internal standard method avoided the need to inject an accurately measured volume of solution on to the column. Phentermine response was not measured directly but always relative to a known amount of internal standard.

Urinary Excretion Trials

Four healthy male volunteers aged 28 - 38 years participated in these experiments. Each subject received an oral dose of 12.5 mg of phentermine hydrochloride (10 mg base) in 10 ml aqueous solution half an hour after a light breakfast. The same subjects were also given one tablet of each drug formulation in a cross-over design. At least two weeks were allowed between each experiment. The oral administration of ammonium chloride (as 0.5 g enteric coated tablets) was used to maintain an acidic urine pH. A typical dosage regimen was 1.0 g of ammonium chloride every four hours taken on the day before the trial and 1.0 G at every three hours during the period of trial.

The urine samples were collected every hour for the first six hours, and at four to six hour intervals during a 60 hour period. Urine volumes were measured for each sample, and urinary pH was measured with a pH meter[a] as soon

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[a] Beckman Expandomatic pH meter, Beckman Instrument Co. Inc., Fullerton, California

as possible after urine collection (within 12 hours).

In order to determine the selectivity of the analytical procedure to determine unchanged drug and not its metabolites under the acidic pH conditions, one subject (GP) was given a 12.5 mg dose of phentermine hydrochloride. The urine was maintained acidic by the administration of ammonium chloride as described earlier. The urine was collected at the end of four hours. A 5 ml sample was analysed for the phentermine content using the GLC technique. Another 5 ml sample of the urine was hydrolyzed by acidifying it with 1N sulphuric acid to pH below 1, and heating it for three hours on a water bath. The total phentermine was extracted using the established procedure and phentermine content was determined. The comparisons of the peak areas for the hydrolyzed and unhydrolyzed samples were made.

pH CHANGES IN VARIOUS FLUIDS

In order to determine the ability to change the pH of various dissolution media, one tablet of formulation II was dissolved in 600 ml of the dissolution media. pH readings were recorded before and after the dissolution process. To ensure the complete dissolution of the drug, the contents were agitated for three hours on a magnetic stirrer. The pH readings were recorded using a pH meter.

The pH changes were measured in distilled water, simulated gastric fluid (209), simulated intestinal fluid

(210), hydrochloric acid solution pH 1.2, sodium hydroxide solution pH 7.5, and phosphate buffer pH 7.5 (212).

DISSOLUTION STUDIES

Three dissolution methods have been used to determine the dissolution behaviour of each of the phentermine tablet formulations. The different dissolution methods were chosen in such a manner as to achieve varying degrees of agitation, intensity and mixing.

Rotating Flask Method

The apparatus used was essentially the same as reported by Gibaldi and Weintraub (41) with slight modifications. The apparatus consists of a spherical glass flask of two liter capacity which is supported in a constant temperature water bath. The globe is supported by two glass tubes fused to its sides, which forms the horizontal axis of the sphere. The assembly is placed on a smooth bearing mounted on the sides of the water bath. One support is coupled to a variable speed motor by means of a chain drive which provides rotation about the horizontal axis. A rotational speed of 1.2 r.p.m. was used. The other support was used as a sample port to permit introduction of the dosage form and periodic withdrawal of samples.

Six hundred millilitres of the dissolution medium was introduced into the flask and the solution was allowed to equilibrate at 37°. One tablet was then introduced into the

flask through the sample port. The samples were withdrawn periodically at suitable intervals for 90 minutes using a glass syringe to which a length of plastic tube was connected. Immediately after withdrawing the sample, the same volume of dissolution medium was added to the flask to maintain a constant volume. An 'infinity' time sample was withdrawn after stirring the entire content of the flask on a magnetic stirrer for four hours. The samples were analyzed using the gas-liquid chromatographic technique. Two runs were carried out for each formulation.

Distilled water, simulated gastric fluid, simulated intestinal fluid, hydrochloric acid solution (pH 1.2), and phosphate buffer (pH 7.5) were used as the dissolution media.

Rotating Basket Method

The rotating basket method to study the dissolution of solid dosage forms is a widely used method and is official in N.F. XIII (44) and U.S.P. XVIII (45). The assembly[a] consisted of a water bath, a covered 1000 ml vessel made of Pyrex glass, a cylindrical stainless steel basket, and a variable speed motor. The rotating basket consisted of a cylinder 3.6 cm in height and 2.5 cm in diameter, the sides and bottom of which are 40 mesh stainless steel cloth. A 6 mm X 30 cm stainless steel rod, attached to a 2.5 cm plate and three spring clips was used to hold the basket.

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[a] Hanson Research Corporation, Northridge, California

The stirring rod of the rotating basket assembly was placed through the center hole of the vessel cover and was centered by suitable means so as to permit smooth rotation and to prevent wobbling. The basket was immersed to a point of two cm from the bottom of the flask.

Nine hundred millilitres of distilled water was introduced into the dissolution vessel, and was allowed to equilibrate at 37°. One tablet was introduced into the basket, and the basket was then lowered into the solution. At regular time intervals a sample was withdrawn. Immediately after withdrawing the sample, the same volume of the dissolution medium was introduced into the vessel to maintain the constant volume. All the dissolution studies were conducted at a rotation speed set at 50 r.p.m. An 'infinity' time sample was obtained as described for the rotating flask method. Two runs were carried out for each formulation.

U.S.P. Tablet Disintegration Apparatus

The apparatus has already been described previously under the 'Disintegration Test'; 900 ml of distilled water at 37° was used as a dissolution medium. One tablet was placed in one of the tubes and the disk was placed over the tablet. The samples were withdrawn at regular time intervals. The same volume of the dissolution medium was replaced after withdrawing the sample. Two runs were performed for each formulation.

Analytical Method

The analytical method employed for the determination of phentermine in the dissolution media was essentially the same as used in urine analysis excepting for a few minor changes. It was noticed that an interfering peak appeared near the chlorphentermine peak on the GLC chromatogram. This peak was found to be due to the presence of light mineral oil in the tablets. To eliminate this peak, the solution was made acidic to pH below 1 by the addition of about 0.5 ml of hydrochloric acid solution (5N). The solution was extracted twice with 2.5 ml of ether. The ether extracts were discarded. The rest of the extraction procedure was the same as that described for the urine analysis. A calibration curve was prepared from solutions containing 10 - 100 mcg phentermine and 50 mcg chlorphentermine in distilled water.

Some of the samples for the dissolution studies were analyzed under different GLC conditions. A Hewlett-Packard 5700 A Gas Chromatograph[a] with dual flame ionization detectors equipped with a strip chart recorder[b] and a disc integrator[c] was used. The temperature programming method was employed between 130° and 160° at the rate of 5° min^{-1}.

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[a] Hewlett-Packard, San Diego, California

[b] Model 7127A, Hewlett-Packard, San Diego, California

[c] Disc Instruments, Inc., California

The chromatographic column of 1/2 inch coiled glass column, six feet in length and packed with 3% OV-17 on Chrom W-AW DMCS (HP) was used. The injection port and detector temperatures were maintained at 200°. Helium was used as the carrier gas at the rate of 60 ml min^{-1} with an inlet pressure of 40 psig. The air and hydrogen pressures were 28 (298 ml min^{-1}) and 25 (68 ml min^{-1}) psig respectively. The peak areas of phentermine and chlorphentermine (internal standard) were measured with the help of the disc integrator. Using these conditions, a calibration curve was prepared from the solutions containing 5 - 70 mcg phentermine and 50 mcg chlorphentermine in distilled water.

In order to measure the precision of the analytical procedure, a solution containing 50 mcg of each phentermine and chlorphentermine was taken and extraction procedures were carried out. Ten determinations for the phentermine content were made from the same solution.

BINDING STUDIES

In order to determine whether an interaction occurred between the drug and the tablet diluents, the binding between phentermine and the diluents was investigated, utilizing the equilibrium dialysis method of Patel and Foss (63). There are many instances where it has been found that drug diluent binding caused generic inequivalence in solid dosage forms. Monkhouse and Lach (213) have discussed these

implications in their recent review article.

Seamless cellulose membrane[a] was placed in distilled water and heated to 90° for one hour, then washed several times with distilled water. The dialysis cells consisted of two plexiglass blocks, each containing a cylindrical cavity. The cells were assembled by placing a four-inch square piece of cellulose membrane over the cavity and then the blocks were clamped together. Ten millilitres of distilled water containing 12.5 mg phentermine hydrochloride was placed in one cavity (side I). An equal volume of distilled water was placed in the other cavity (side II). Two hundred and fifty milligrams of the diluent was placed in the cavities on each side of the membrane. The cells were then agitated on a shaker bath at 37° until the equilibrium was established. The time required to attain equilibrium was determined by using a control cell containing 10 ml of phentermine hydrochloride solution on side I and an equal volume of distilled water on side II. Identical concentrations of the drug on both sides of the membrane were observed at the end of the equilibrium period. Equilibrium was established after 24 hours.

In another experiment, a 10 ml saturated solution of calcium phosphate containing 12.5 mg phentermine hydrochloride was placed on one side of the membrane and 10 ml of

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[a] Fisher Scientific Co. Ltd., Pittsburgh, Pennsylvania

distilled water was placed on the other side.

At the end of the equilibrium time, 50 µl aliquots were quantitatively removed from both sides of the membrane and analyzed for the phentermine content using the GLC technique. Three equilibrium dialysis cells were used for each drug-diluent binding study.

ANALOG COMPUTER SIMULATION

Using an electronic analog computer, the urinary excretion data for phentermine in the solution and tablet form was examined in order to evaluate pharmacokinetic parameters. In addition, these studies also provided the information as to whether one compartmental model could mathematically describe the pharmacokinetics of phentermine after its administration in various dosage forms. The analog computer simulates the assumed physiological models by an electrical network. The computer uses an electrical voltage to represent the drug quantity, and can perform mathematical operations, such as summation, multiplication and integration by groups of electronic components (214,215).

A PACE TR-20[a] analog computer was used together with a X-Y recorder[b]. Systematic variation of the rate potentiometer was made within the possible limits of the chosen compartmental models in an effort to fit the computer

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[a] Electronic Associates Ltd., Princeton, New Jersey

[b] Electronic Associates Ltd., Princeton, New Jersey

generated curves to the experimental data previously plotted on recorder paper. The experimental data and compartmental model could be evaluated as the best possible fit, and the rate constants determined from the potentiometer settings.

Pharmacokinetic Model

The following assumptions were made for the use of the one compartmental model to investigate the kinetics of absorption, distribution, and elimination of phentermine administered as various dosage forms under acidic urine conditions:

(1) the rate of urinary excretion of the drug is proportional to its concentration in plasma,

(2) drug transfer from one compartment to another is irreversible,

(3) the rate of transfer of drug from one compartment to another is directly proportional to the amount of drug in that compartment, i.e. drug release, absorption, and excretion follow apparent first order kinetic processes having units of reciprocal time,

(4) compartments are uniform and homogeneous throughout the transfer process,

(5) there is no decomposition of the drug at the absorption site nor diffusion of the drug from the blood into the stomach,

(6) the rate constant for drug absorption is unchanged along the gastrointestinal tract,

(7) excretion of unchanged drug by pathways other than via the kidney is negligible, and little or no metabolism of the drug occurred,

(8) absorption and elimination rate constants are independent of dosage forms

A pharmacokinetic model was proposed to describe the kinetics of absorption, distribution, and excretion of phentermine in man after oral administration of the drug in solution and in tablet form under the controlled acidic urine condition (see Figure 1).

Based on this model, the following differential equations may be written:

$$\frac{dA}{dt} = -K_a A \qquad \text{(Equation 11)}$$

$$\frac{dB}{dt} = + K_a A - K_u B \qquad \text{(Equation 12)}$$

$$\frac{du}{dt} = + K_u B \qquad \text{(Equation 13)}$$

where A = the amount of drug in the gastrointestinal tract

B = the amount of drug in the blood compartment

u = the amount of drug in the urine

K_a = the rate constant for the absorption of drug from the gastrointestinal tract into the blood

K_u = the rate constant for the excretion of drug from blood compartment into the urine

t = time after ingestion of the dose

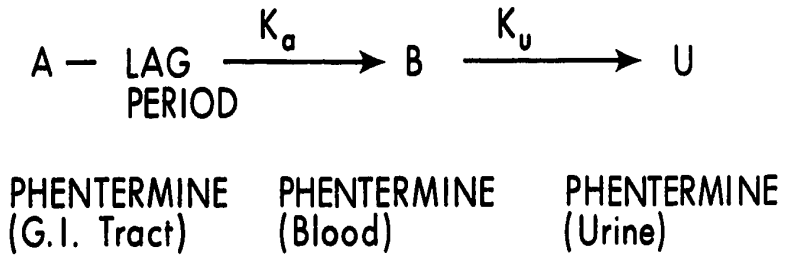

Figure 1: Pharmacokinetic Model for Phentermine Dosage Forms in Man Following oral Administration

The analog computer program for the solution of the differential equations is shown in Figure 2. The curve was fitted by manually setting the abscissa zero on the X-Y recorder, and lag time estimated from the intercept on the abscissa scale.

DIGITAL COMPUTER SIMULATION

The model shown in Figure 1 was programmed to fit the urinary excretion data using a digital computer program 'NONLIN' (201). An IBM 360 computer[a] with Fortran IV G compiler was used. A Subroutine 'DFUNC' was written to describe the model and was added to main program 'NONLIN'.

The integrated form of Equation 13 was used in Subroutine 'DFUNC'. Equation 14 represents the integrated form of Equation 13.

$$U(t) = A_o \left[1 + \frac{1}{k_a - k_u} \left(K_u e^{-k_a t} - K_a e^{-k_u t} \right) \right] \quad \text{(Equation 14)}$$

where $U(t)$ = the cumulative amount of phentermine in urine at time t,

A_o = amount of phentermine in gastrointestinal tract at zero time.

Initial estimates of A_o, k_a, and k_u as obtained by the analog computer were supplied for each run. The steps of Subroutine 'DFUNC' are shown in Figure 3.

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[a] Computing Center, University of Alberta, Edmonton, Alberta

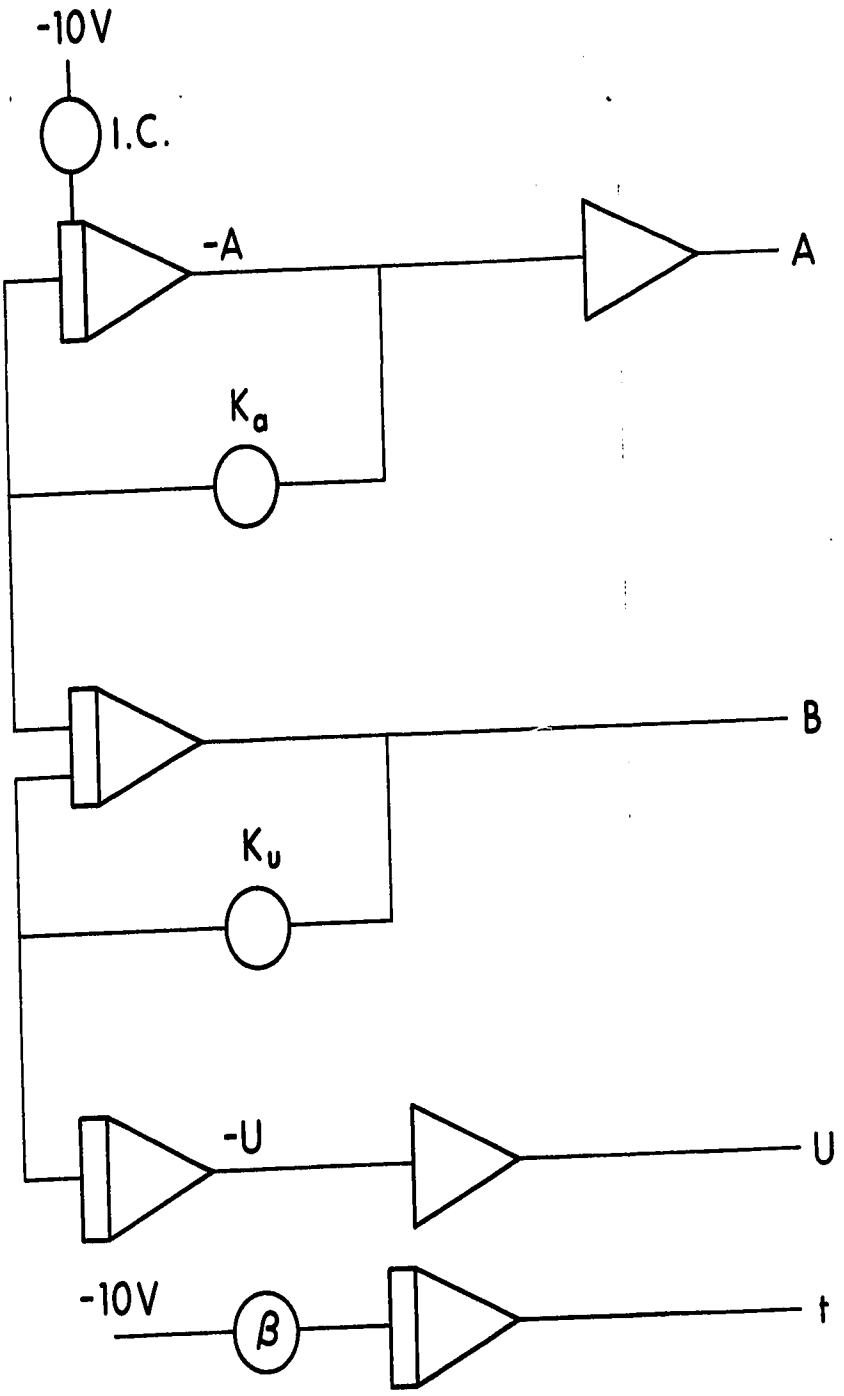

Figure 2: Analog Computer Program for Model in Figure 1

```
      SUBROUTINE DFUNC(T,I,J,K,P,VAL)

      COMMON NP,NC,NIV,NDE,NIT,NTP,PM,IP,IT,SS,WS
      COMMON PL(32),CON(8),IW(10),NPT(11),Y(10),IDIG(10)
      COMMON NAME,LPLOT,LDEG,TEST,DEL
      COMMON YOBS(400),X(800),DV(400),W(400),YCALC(400)
      DIMENSION P(1),VAL(1),NAME(10)

            THE FOLLOWING STATEMENTS DEFINE THE FUNCTION

      P1=P(1)
      P2=P(2)
      A0=P(3)
      P21=P1-P2
      XT=X(1)
      E1=P2*EXP(-P1*XT)
      E2=P1*EXP(-P2*XT)
      T=A0*(1.+((E1-E2)/P21))
      IF(IP-9)40,30,40
30    TK1=-ALOG(0.5)/P1
      TK2=-ALOG(.5)/P2
      WRITE(6,1)TK1,TK2
1     FORMAT('  TK1 =',F6.2,'     TK2 =  ',F6.2)
40    RETURN
      END
```

Figure 3: Subroutine 'DFUNC' for NONLIN

RESULTS

TABLET WEIGHT VARIATION TEST

All four tablet formulations were subjected to the U.S.P. weight variation test. A slight variation was found among the tablets, but it was well within the U.S.P. limits for a 250 mg tablet (208).

CONTENT UNIFORMITY TEST

Ten tablets from each formulation were individually analyzed using gas-liquid chromatographic procedure. The mean phentermine content for each tablet formulation along with the standard deviations is presented in Table III.

Table III

Drug Content of Phentermine Tablet Formulations

Formulation No.	Phentermine Content[a] (mcg)
I	9824 ± 21.7
II	10006 ± 28.2
III	9982 ± 30.7
IV	9799 ± 49.3

[a] Calculated as phentermine base

DISINTEGRATION TEST

The results of the U.S.P. disintegration test in different media are presented in Table IV.

Table IV

Disintegration Times of Phentermine Tablet Formulations

Formulation No.	Disintegration Time[a], min		
	Distilled Water	Simulated Intestinal Fluid U.S.P.	Simulated Gastric Fluid U.S.P.
I	8.00 ± 0.45	6.55 ± 0.51	9.22 ± 0.22
II	4.52 ± 0.60	5.30 ± 0.50	5.19 ± 0.16
III	6.51 ± 0.61	9.03 ± 0.81	8.50 ± 0.43
IV	11.50 ± 0.40	12.71 ± 0.63	12.63 ± 0.51

[a] average of three determinations with standard deviation

URINARY EXCRETION STUDIES

Analysis of Phentermine in Urine

The extraction of phentermine from urine was found to be very efficient giving a recovery of 97 - 100% (see Table V).

The reproducibility of the extraction procedure was obtained by extracting 12 urine samples, each containing 10 mg phentermine, and found to be 10 ± 0.5 mg.

Table V

Recovery of Phentermine from Blank Urine

Phentermine (mcg)	Recovered[a] (mcg)	Recovery (%)
1.0	0.98	98.0
2.0	1.94	97.0
5.0	5.00	100.0
10.0	9.80	98.0
20.0	19.60	98.0
30.0	30.00	100.0
40.0	39.80	99.5
60.0	59.20	98.6

[a] average of four determinations

No deterioration of phentermine in urine occurred when the solutions were stored at 4°C for two weeks.

There were no interfering peaks found in the blank urine samples having the same retention times as phentermine or chlorphentermine.

Figure 4 shows a typical chromatogram of a urine extract containing phentermine and internal standard, chlorphentermine. The retention times of the drug and that of the internal standard were found to be 3 and 6.4 minutes respectively.

Figure 5 illustrates a calibration curve for

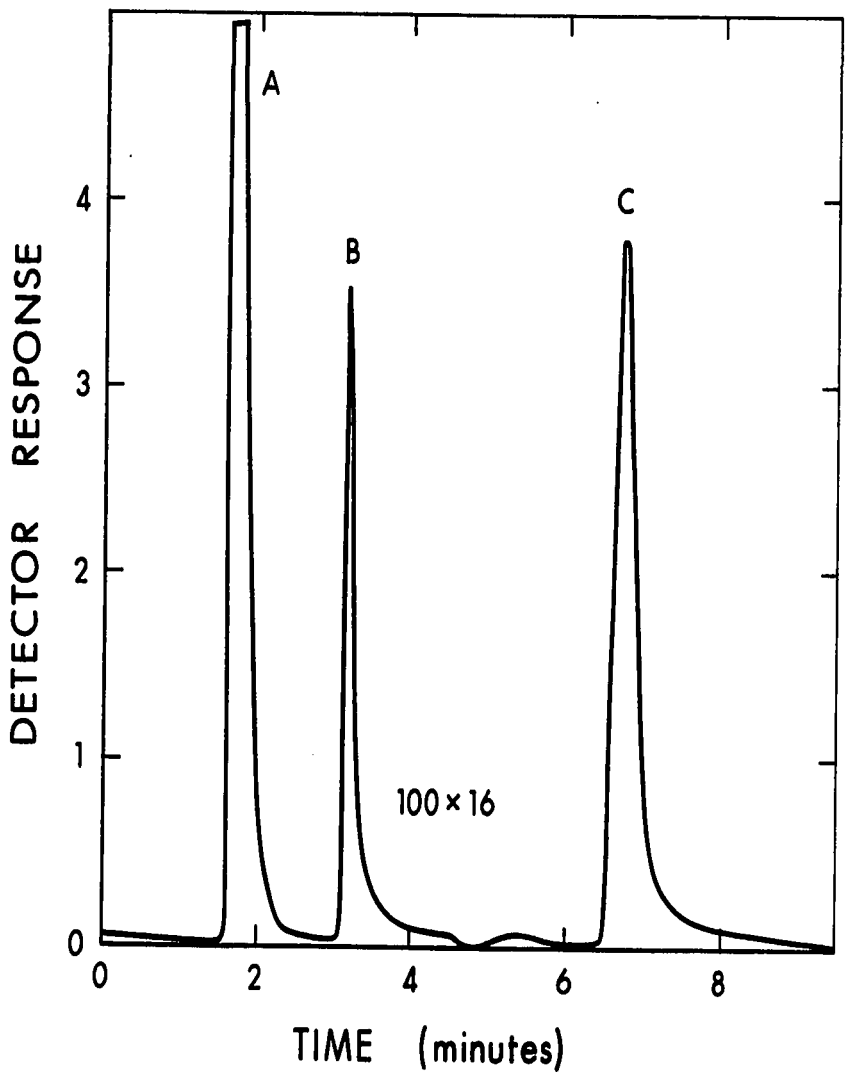

Figure 4: Typical Gas Chromatogram of Urine Extract Containing Phentermine

A = solvent (ether)
B = Phentermine
C = Chlorphentermine (internal standard)
Instrument: Perkin-Elmer 990 Gas Chromatograph

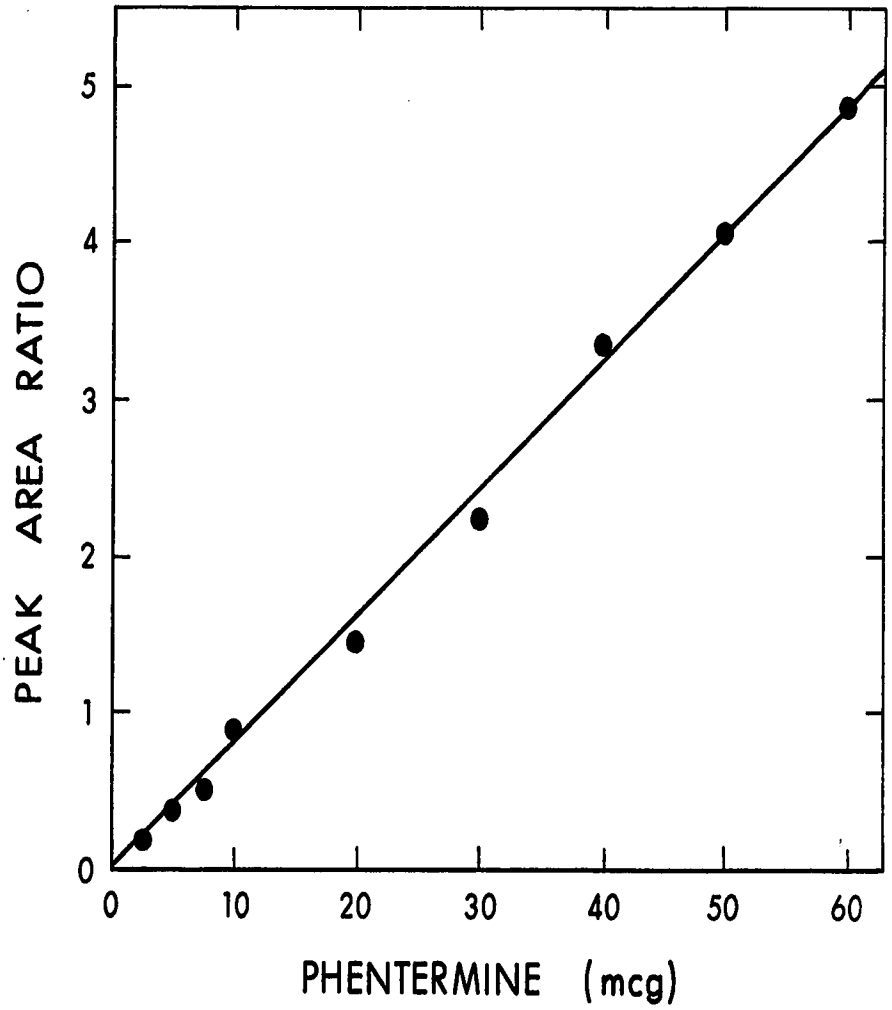

Figure 5: GLC Calibration Curve for Phentermine in Urine

phentermine in urine. The data for the calibration curve are presented in Table VI. The curve is linear over the range 2.5 - 60 mcg of phentermine having a slope of 0.0821 and intercept of -0.0761. Calibration curves prepared at later dates did not differ significantly.

Table VI

Data for the Calibration Curve for Phentermine in Urine

Phentermine (mcg)	Peak Area Ratio[a]
2.5	0.149 ± 0.316
5.0	0.311 ± 0.108
7.5	0.489 ± 0.112
10.0	0.863 ± 0.096
20.0	1.466 ± 0.034
30.0	2.227 ± 0.052
40.0	3.305 ± 0.049
50.0	4.041 ± 0.036
60.0	4.875 ± 0.038

[a] average of three determinations with standard deviation

The optimal air and hydrogen pressures for maximum response of drug and internal standard were found to be 30 and 25 psig respectively.

Urinary Excretion Trials

Figure 6 represents the urinary excretion profile of phentermine following an oral administration of a solution containing a 10 mg dose to one subject (JM). The urinary pH was uncontrolled. Large fluctuations in the urinary excretion rate of the drug was found which may be attributed to the changes in urinary pH and urine volume. Urinary pH appeared to be the major factor influencing the excretion rate of the drug. Under the controlled urine pH condition, the fluctuations in the urinary excretion rate were greatly reduced as shown in Figure 7. The slight fluctuations may be attributed to the changes in urine volume.

The comparison of results between the hydrolyzed and unhydrolyzed samples obtained under controlled acidic urinary pH conditions (subject GP) indicated that the method of analysis is specific to detect the unchanged drug in the urine. It was found that when the urine sample was hydrolyzed, a 12.5% increase in peak area of phentermine was found. Three determinations were carried out.

Figure 8 shows the large differences in the cumulative excretion of phentermine under acidic and uncontrolled urine pH conditions in one subject (JM). The recovery under uncontrolled urine pH conditions was 41.3%, and with urinary pH controlled acid by oral administration of ammonium chloride, the recovery was 75%.

Figures 9 - 12 illustrate the urinary excretion of

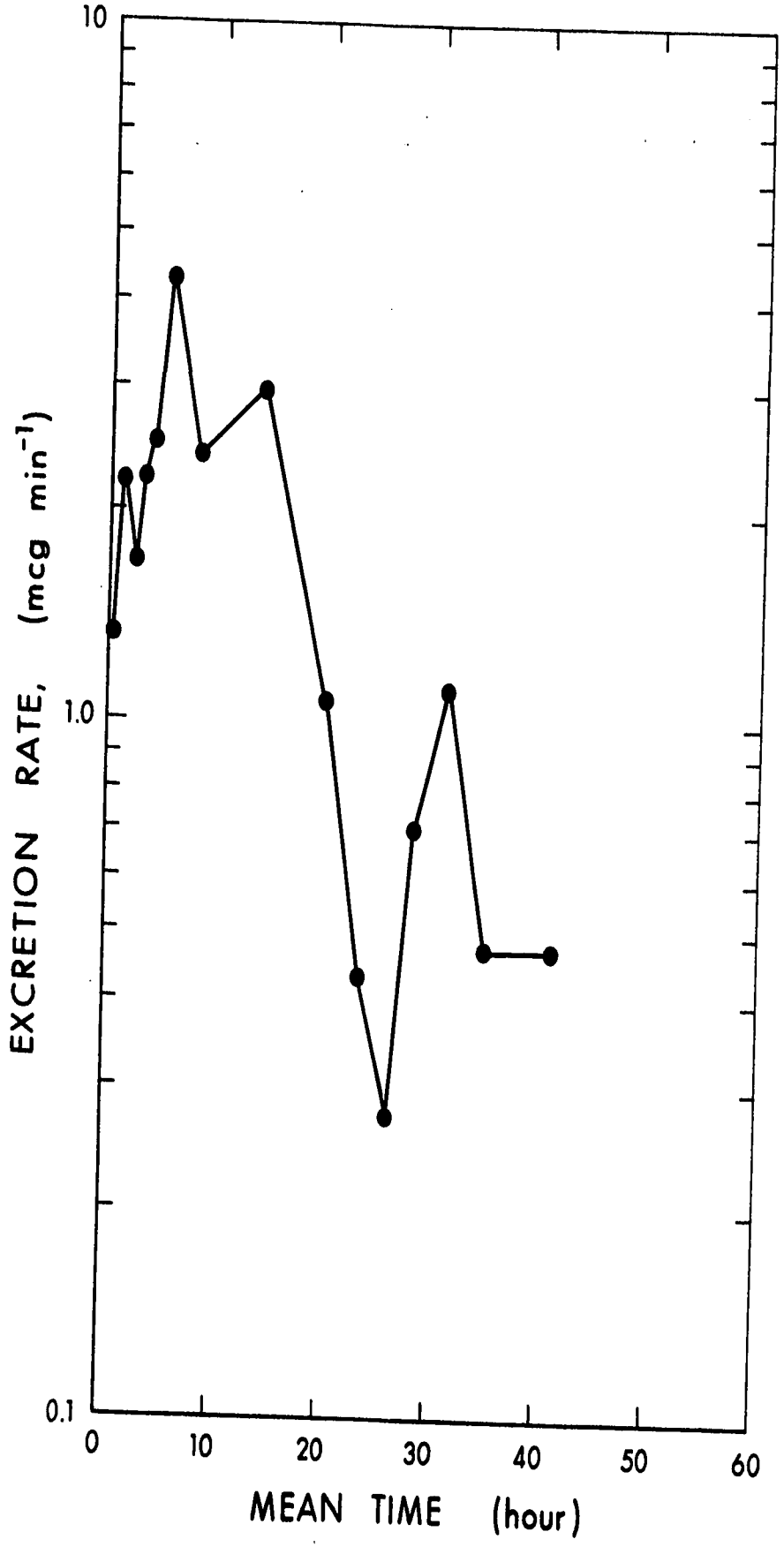

Figure 6: The Urinary Excretion Profile of Phentermine Following Oral Administration of Drug in Solution

Dose: 10 mg; Urine pH uncontrolled; Subject JM

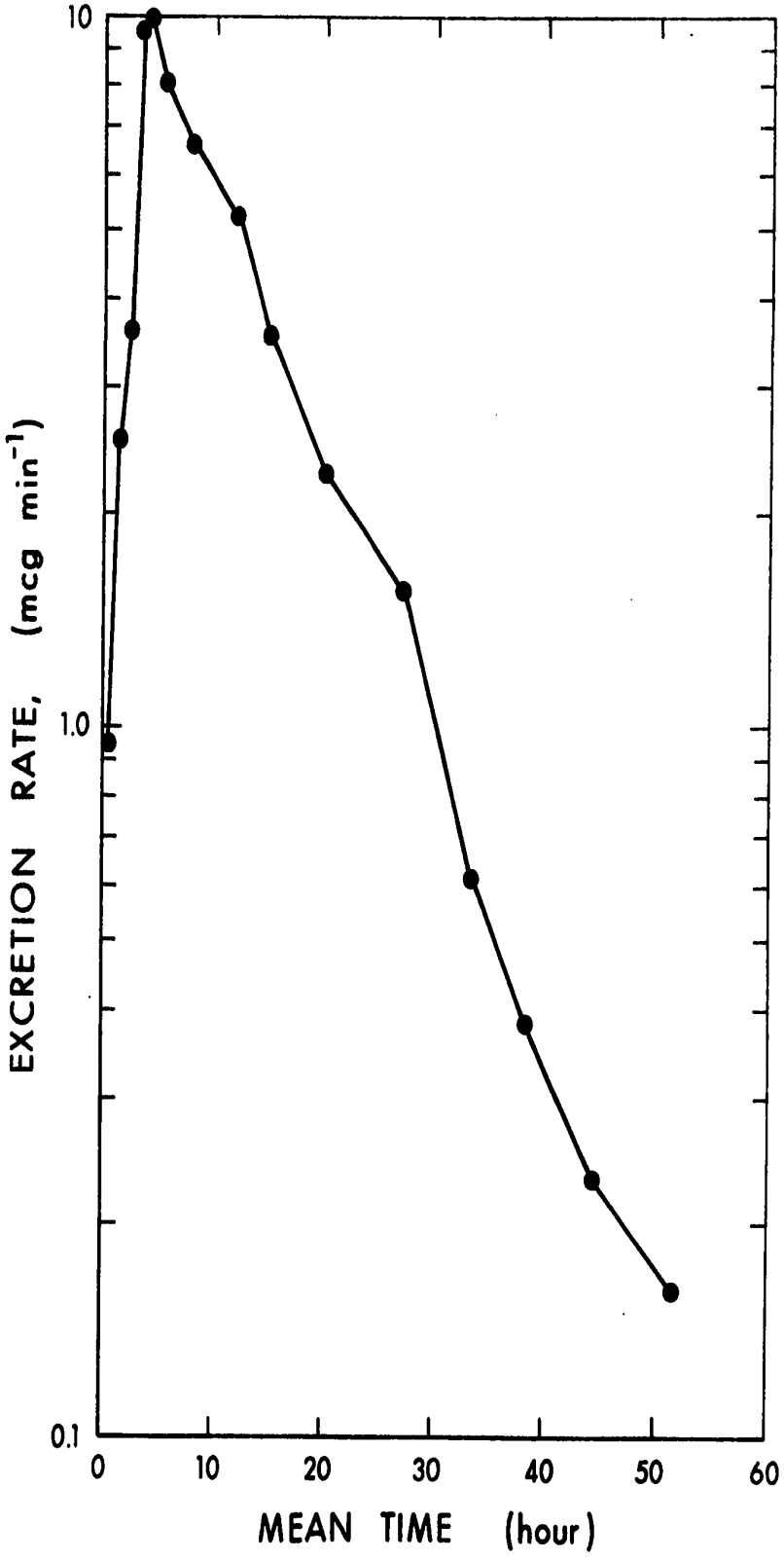

Figure 7: The Urinary Excretion Profile of Phentermine Following Oral Administration of Drug in Solution

Dose: 10 mg; Urine pH controlled; Subject JM

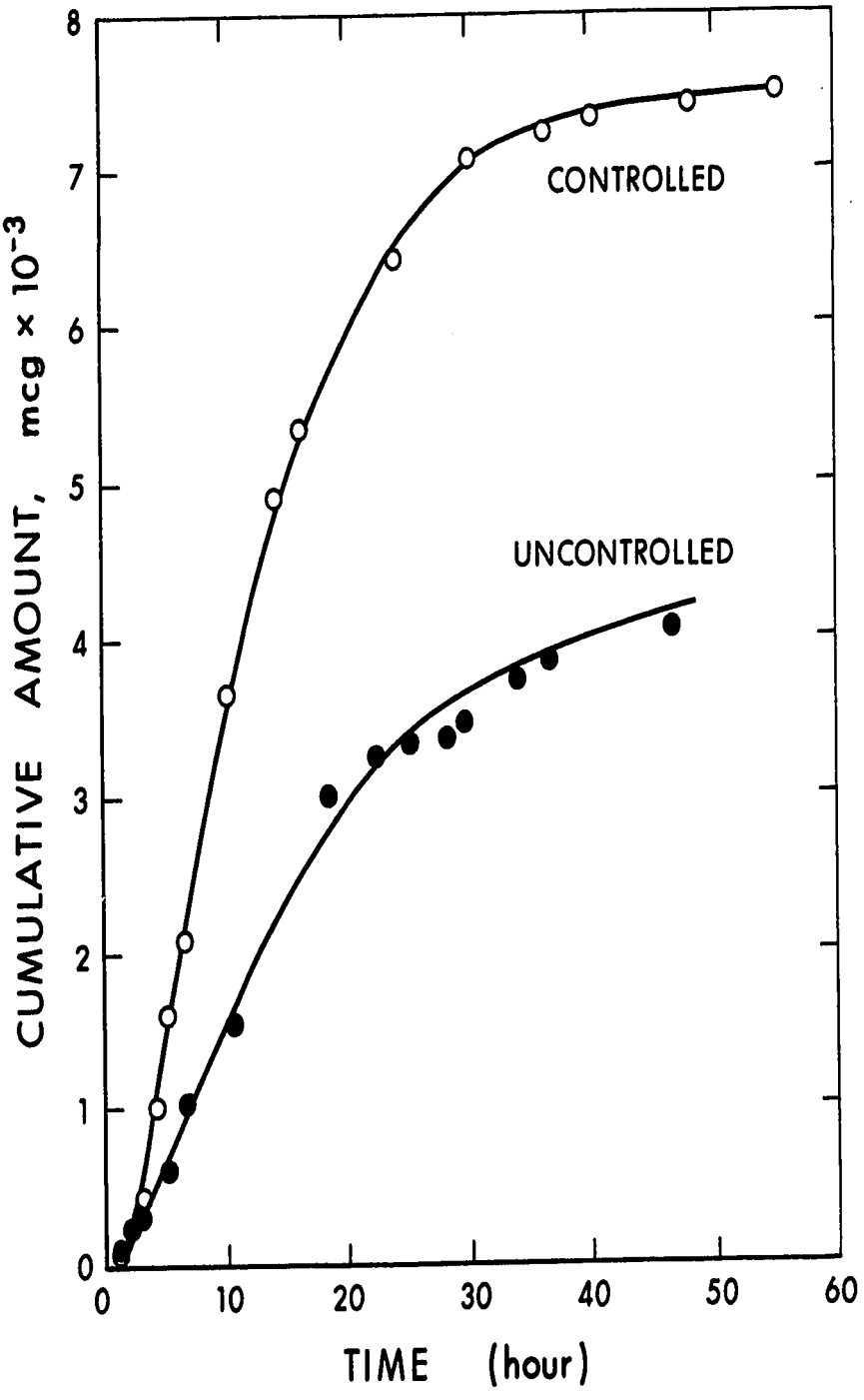

Figure 8: Cumulative Amount of Phentermine Excreted Following Oral Administration of Phentermine Solution Under Controlled and Uncontrolled Urinary pH; Dose: 10 mg; Subject JM

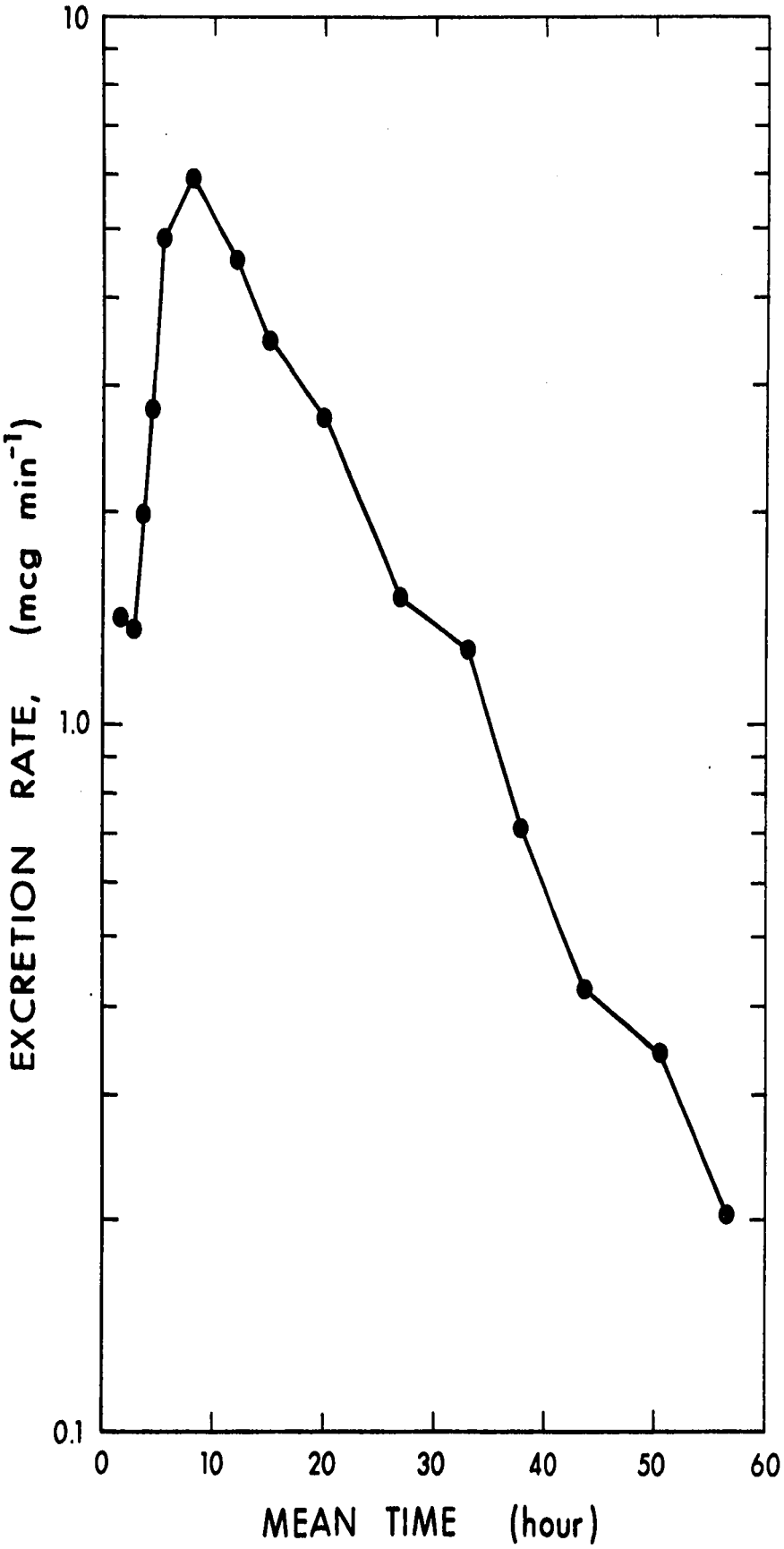

Figure 9: The Urinary Excretion Profile of Phentermine Following Oral Administration of One Tablet of Formulation III; Urine pH controlled; Subject GP

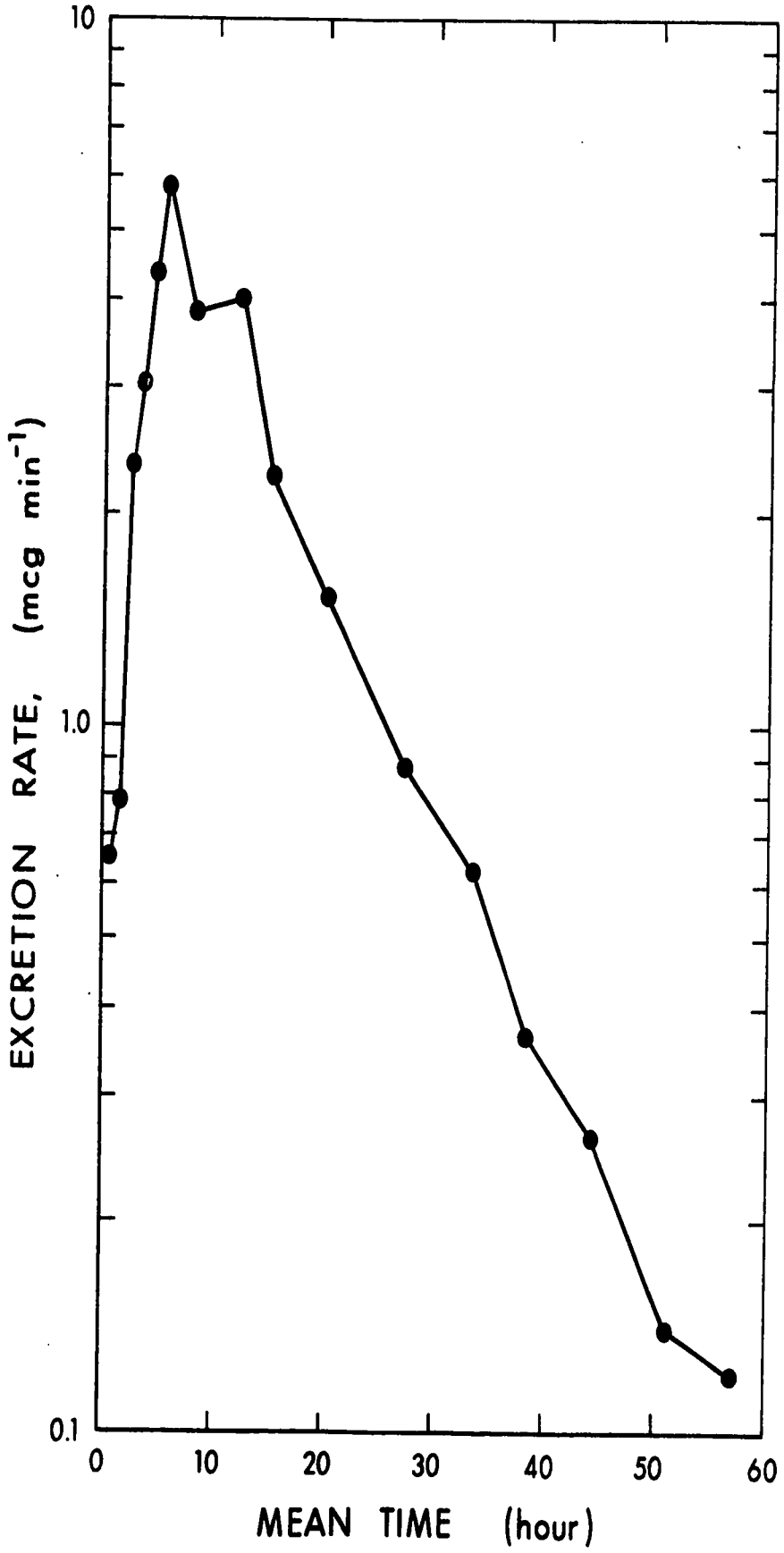

Figure 10: The Urinary Excretion Profile of Phentermine Following Oral Administration of One Tablet of Formulation I; Urine pH controlled; Subject HC

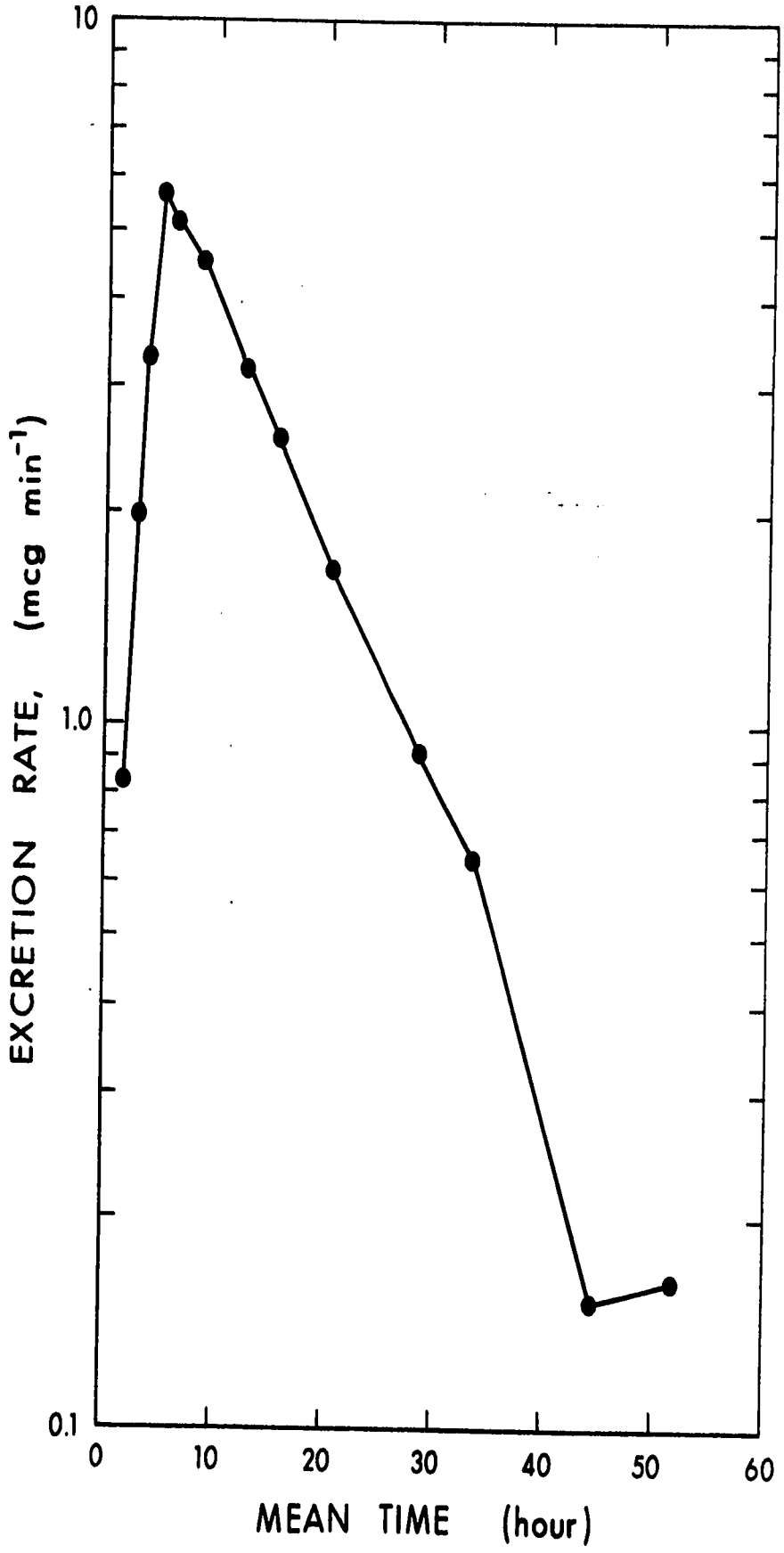

Figure 11: The Urinary Excretion Profile of Phentermine Following Oral Administration of One Tablet of Formulation II; Urine pH controlled; Subject SM

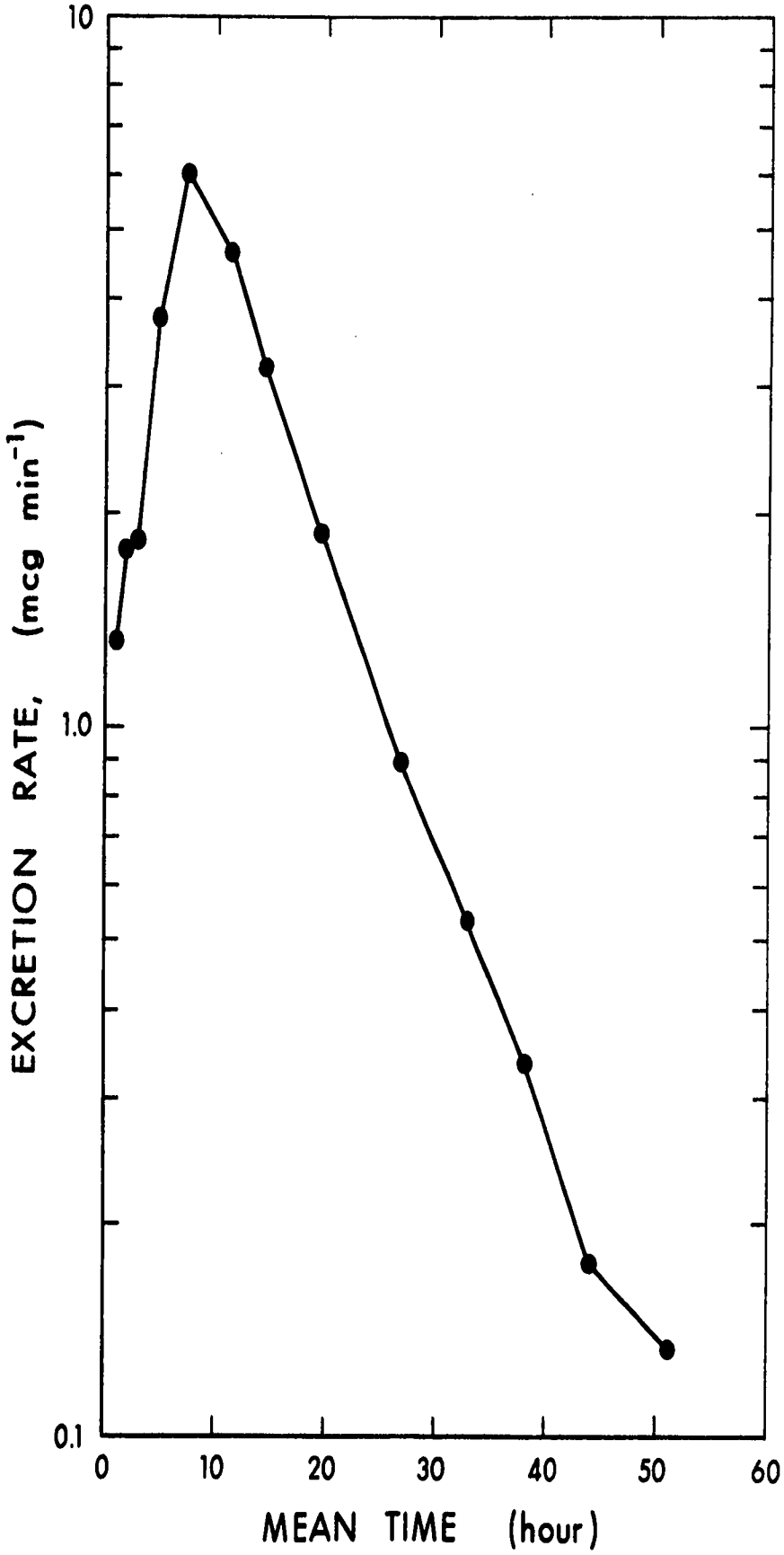

Figure 12: The Urinary Excretion Profile of Phentermine Following Oral Administration of One Tablet of Formulation IV; Urine pH controlled; Subject JM

the drug following the oral administration of 10 mg of phentermine in solution and tablet form. The complete data for the urinary excretion study are tabulated in the Appendix.

PERCENT ABSORBED-TIME PLOTS

The method of Wagner and Nelson (106,183) was used to construct percent absorbed-time plots for each formulation as derived from the urinary excretion data under controlled urinary pH conditions. The following equation was used:

$$A_T(f) = \frac{1}{k_e} \cdot \frac{dx_u}{dt} + X_u \qquad \text{(Equation 15)}$$

where $A_T(f)$ = fraction of dose absorbed at time t

k_e = elimination rate constant, hr^{-1}

dX_u/dt = urinary excretion rate, mcg hr^{-1}

X_u = cumulative amount of unchanged drug in the urine at time t, mcg

The excretion rate values were determined at the midpoints (mean times) between urine collection intervals. The excretion rate values were then divided by k_e and added to the cumulative amount of the drug excreted up to that time period. In this manner successive values of $A_T(f)$ were calculated until a maximum or asymptotic value of $A_T(f)$ was obtained. The maximum or asymptotic value was then divided

into the successive values of $A_T(f)$ and multiplied by 100 to get the percent absorbed value as a function of time. The graphs of percent unabsorbed against time for each subject are shown in Figures 13 - 16. From the graphs, the intermediate values of various percent absorbed values were read and are presented in Tables VII - X.

DISSOLUTION STUDIES

Calibration Curve

A calibration curve was prepared for phentermine using aqueous solution containing 10 - 100 mcg of the drug and 50 mcg of chlorphentermine (as an internal standard). The data for the calibration curve are presented in Table XI.

Figure 17 shows the calibration curve for phentermine in distilled water using the Perkin Elmer 990 gas chromatograph. The curve is linear over the range of 10 - 100 mcg. The slope of the regression line was found to be 0.0742 with the intercept of 0.0357.

Figure 18 shows a typical gas chromatogram using the Hewlett-Packard gas chromatograph. When a 2 µl sample containing 50 mcg phentermine was injected ten times on to the column, a reproducible result was obtained. The precision of the assay procedure was found to be 50 $\pm$ .93 mcg.

A calibration curve was prepared from solutions containing 5 - 70 mcg phentermine and 50 mcg chlorphentermine in distilled water. The data for the calibration curve

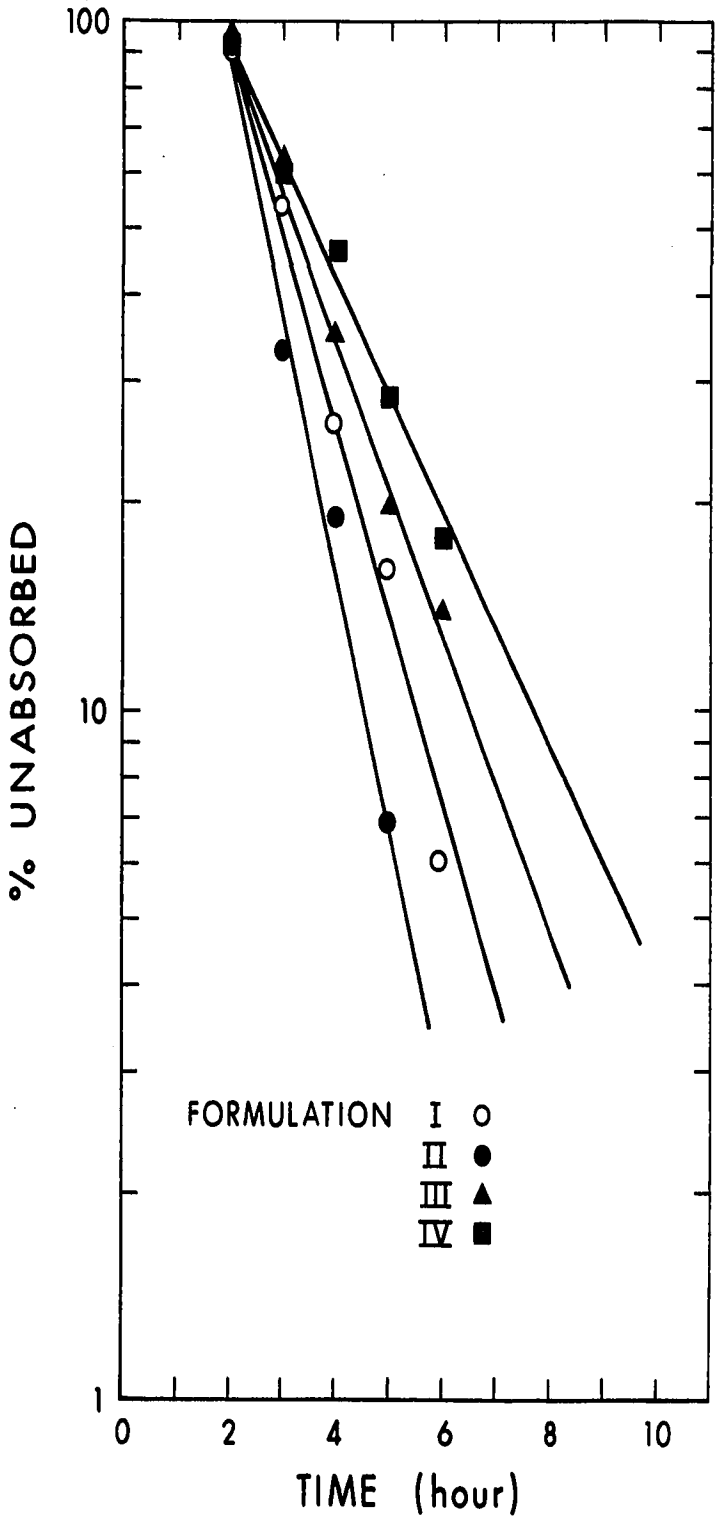

Figure 13: Semilogarithmic Plot of Percent Phentermine Unabsorbed from Tablet Formulations Against Time. Subject JM

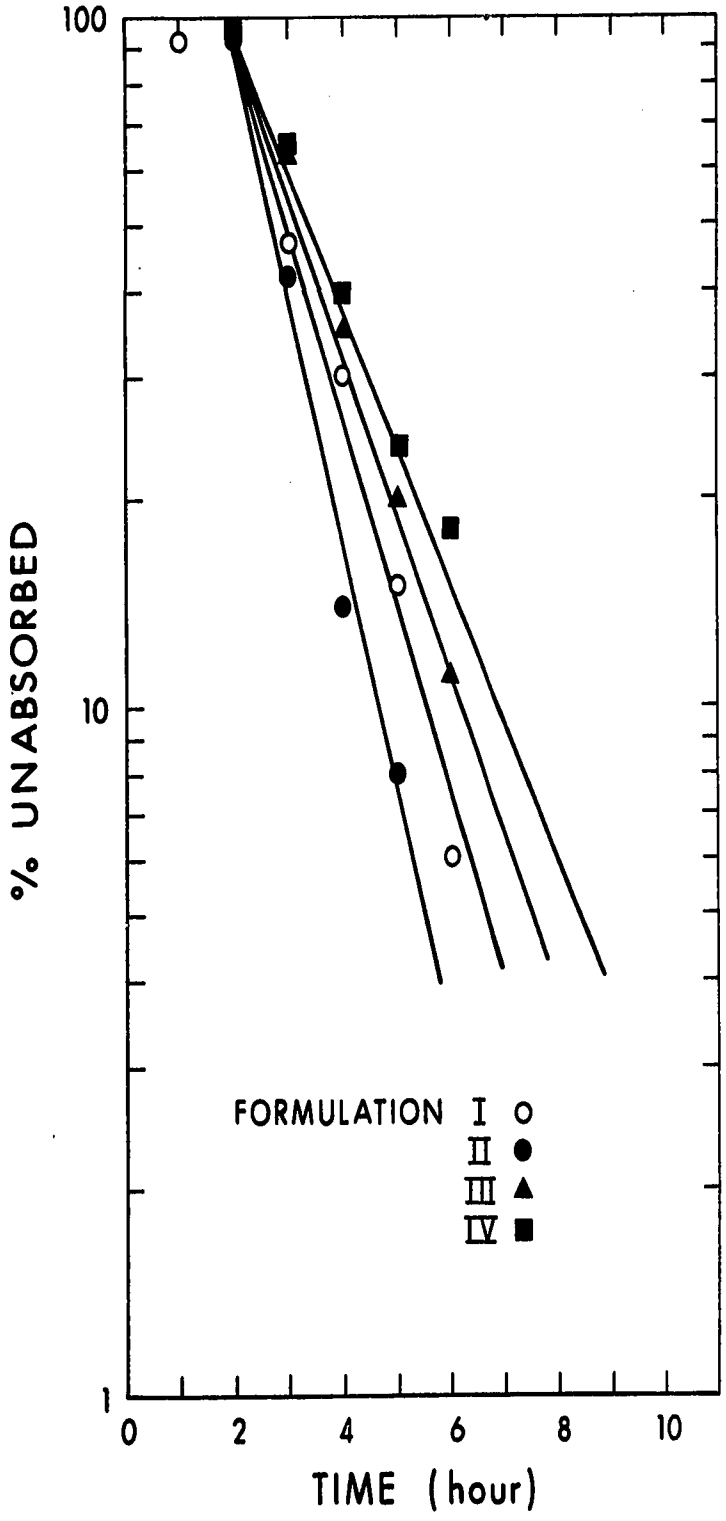

Figure 14: Semilogarithmic Plot of Percent Phentermine Unabsorbed from Tablet Formulations Against Time. Subject HC

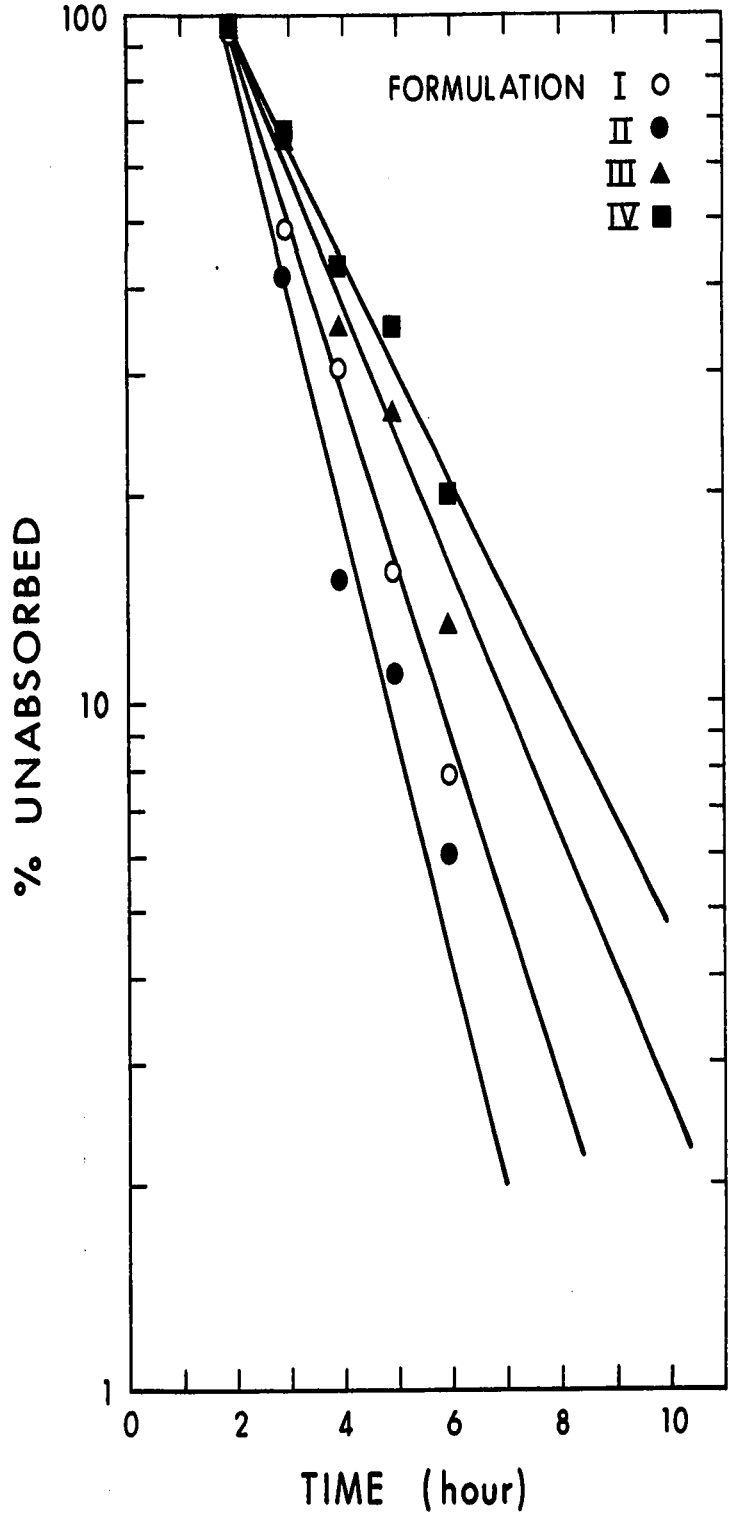

Figure 15: Semilogarithmic Plot of Percent Phentermine Unabsorbed from Tablet Formulations Against Time. Subject SM

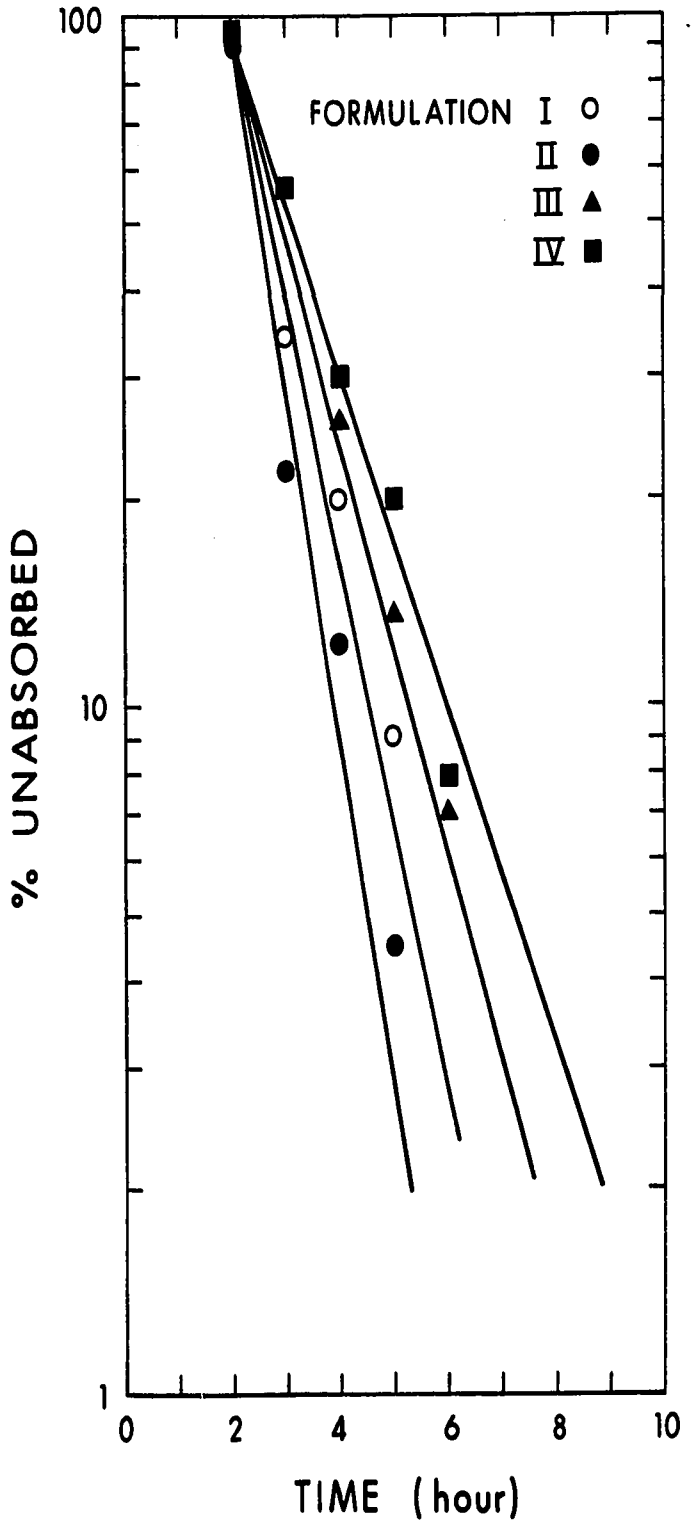

Figure 16: Semilogarithmic Plot of Percent Phentermine Unabsorbed from Tablet Formulations Against Time. Subject GP

Table VII

Percent Absorbed-Time Values for Phentermine Tablet Formulations
Subject - JM

% Absorbed	Time Required for Absorption (hr)			
	Formulation I	Formulation II	Formulation III	Formulation IV
30	2.45	2.30	2.60	2.70
40	2.70	2.50	2.90	3.10
50	3.00	2.70	3.25	3.55
60	3.33	2.95	3.70	4.10
70	3.80	3.30	4.30	4.77
80	4.42	3.75	5.10	5.90

Table VIII

Percent Absorbed-Time Values for Phentermine Tablet Formulations
Subject - HC

% Absorbed	Time Required for Absorption (hr)			
	Formulation I	Formulation II	Formulation III	Formulation IV
30	2.40	2.30	2.50	2.70
40	2.65	2.50	2.80	3.00
50	2.95	2.70	3.17	3.40
60	3.30	3.00	3.57	3.83
70	3.80	3.33	4.10	4.50
80	4.45	3.83	4.90	5.40

Table IX

Percent Absorbed-Time Values for Phentermine Tablet Formulations
Subject - SM

% Absorbed	Time Required for Absorption (hr)			
	Formulation I	Formulation II	Formulation III	Formulation IV
30	2.50	2.30	2.70	2.80
40	2.80	2.50	3.00	3.30
50	3.10	2.70	3.40	3.70
60	3.45	3.00	3.90	4.30
70	3.93	3.40	4.55	5.07
80	4.63	3.93	5.50	6.17

Table X

Percent Absorbed-Time Values for Phentermine Tablet Formulations
Subject - GP

% Absorbed	Time Required for Absorption (hr)			
	Formulation I	Formulation II	Formulation III	Formulation IV
30	2.30	2.22	2.40	2.50
40	2.50	2.33	2.63	2.80
50	2.80	2.50	2.90	3.10
60	2.95	2.70	3.17	3.50
70	3.28	2.97	3.63	4.00
80	3.75	3.30	4.25	4.70

Table XI

Data for the Calibration Curve for Phentermine in Distilled Water Using the Perkin-Elmer 990 Gas Chromatograph

Phentermine (mcg)	Peak Area Ratio[a]
10	0.812 ± 0.1365
20	1.591 ± 0.0186
30	2.157 ± 0.0792
40	3.098 ± 0.1295
50	3.710 ± 0.0835
60	4.455 ± 0.0659
70	5.200 ± 0.0761
80	5.183 ± 0.0459
90	6.730 ± 0.1333
100	7.605 ± 0.1456

[a] average of three determinations with standard deviations

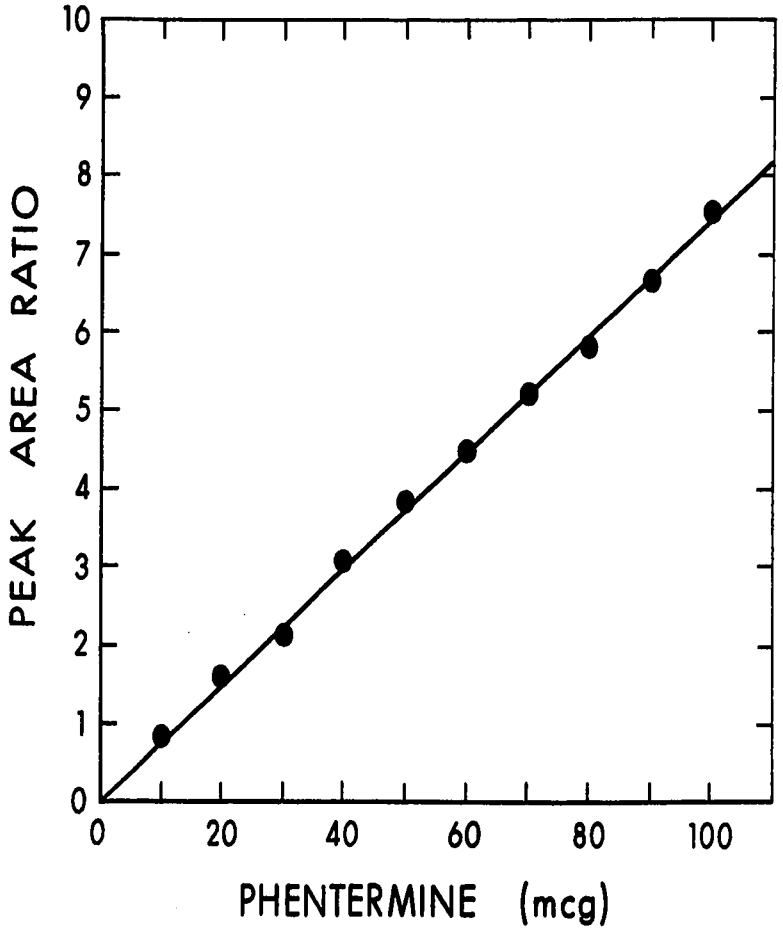

Figure 17: GLC Calibration Curve for Phentermine in Distilled Water using Perkin-Elmer 990 Gas Chromatograph

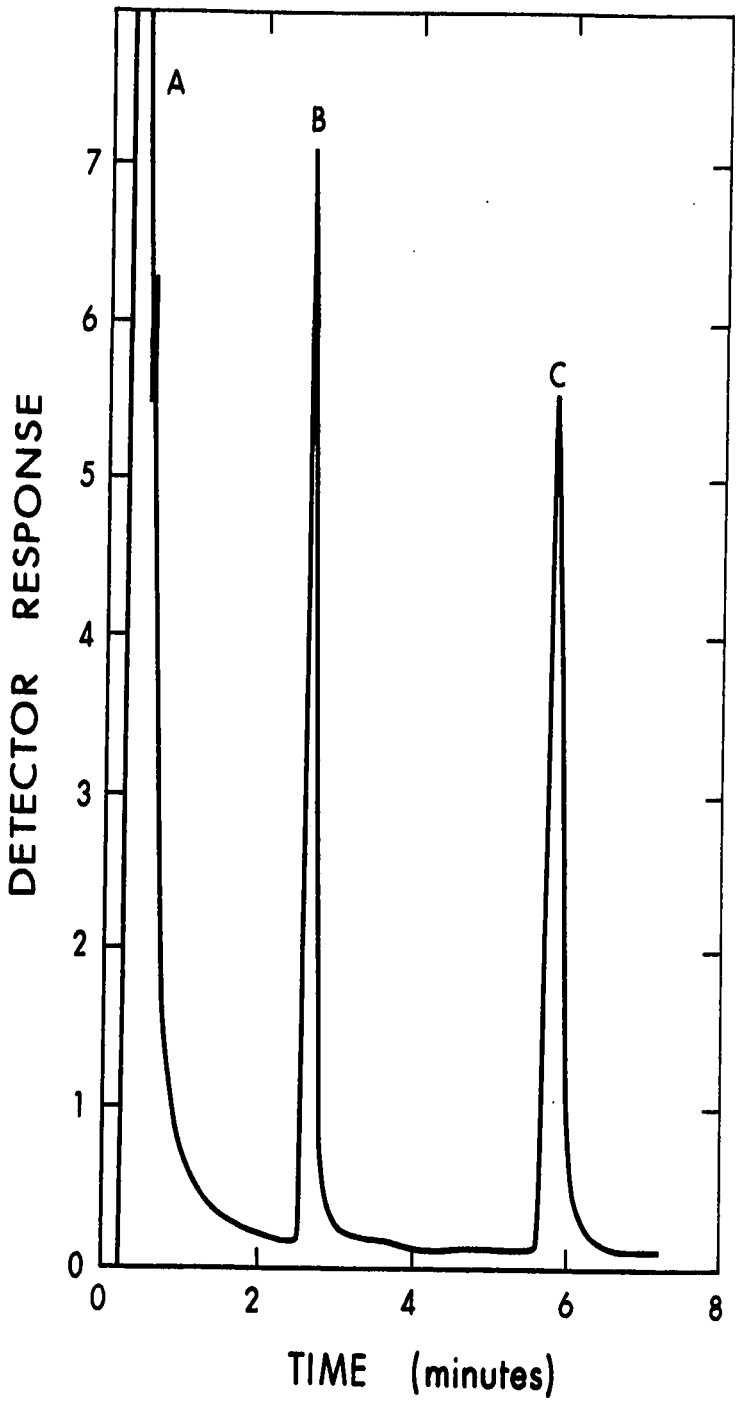

Figure 18: Typical Gas Chromatogram of Phentermine from an Aqueous Solution

A = Solvent (CS_2)
B = Phentermine
C = Chlorphentermine (internal standard)
Instrument: Hewlett-Packard Gas Chromatograph

is presented in Table XII. A linear relationship between the peak area ratios (area of phentermine peak/area of chlorphentermine peak) and the amount of phentermine was obtained. The calibration curve is shown in Figure 19. The slope of the regression line was found to be 0.0326 having the intercept of -0.0149.

Dissolution Studies Using U.S.P. Disintegration Apparatus

The dissolution studies for each tablet formulation were carried out at 37° in distilled water. Three to five millilitre samples were removed at specified time intervals and immediately replaced with an equal volume of distilled water at the same temperature. The samples were analyzed using the GLC method. A cumulative correction was made for the previously removed sample using the method described by Wurster and Taylor (216).

The data for the dissolution studies for each formulation using U.S.P. disintegration apparatus are presented in Tables XIII - XVI. Figure 20 shows the percent dissolved-time plot for each formulation.

Dissolution Studies Using Rotating Basket Apparatus

Using the rotating basket method, the dissolution studies for each tablet formulation were performed. Distilled water at 37° was used as the disintegration medium. The data for each tablet formulation are presented in Tables XVII - XX. The percent dissolved-time plots for each

Table XII

Date for the Calibration Curve for Phentermine in Distilled Water Using the Hewlett-Packard Gas Chromatograph

Phentermine (mcg)	Peak Area Ratio[a]
5	0.123 ± 0.0403
10	0.317 ± 0.1035
15	0.468 ± 0.0629
20	0.661 ± 0.0758
30	0.974 ± 0.1098
40	1.244 ± 0.1261
50	1.704 ± 0.1496
60	1.919 ± 0.0346
70	2.249 ± 0.0337

[a] average of four determinations with standard deviations

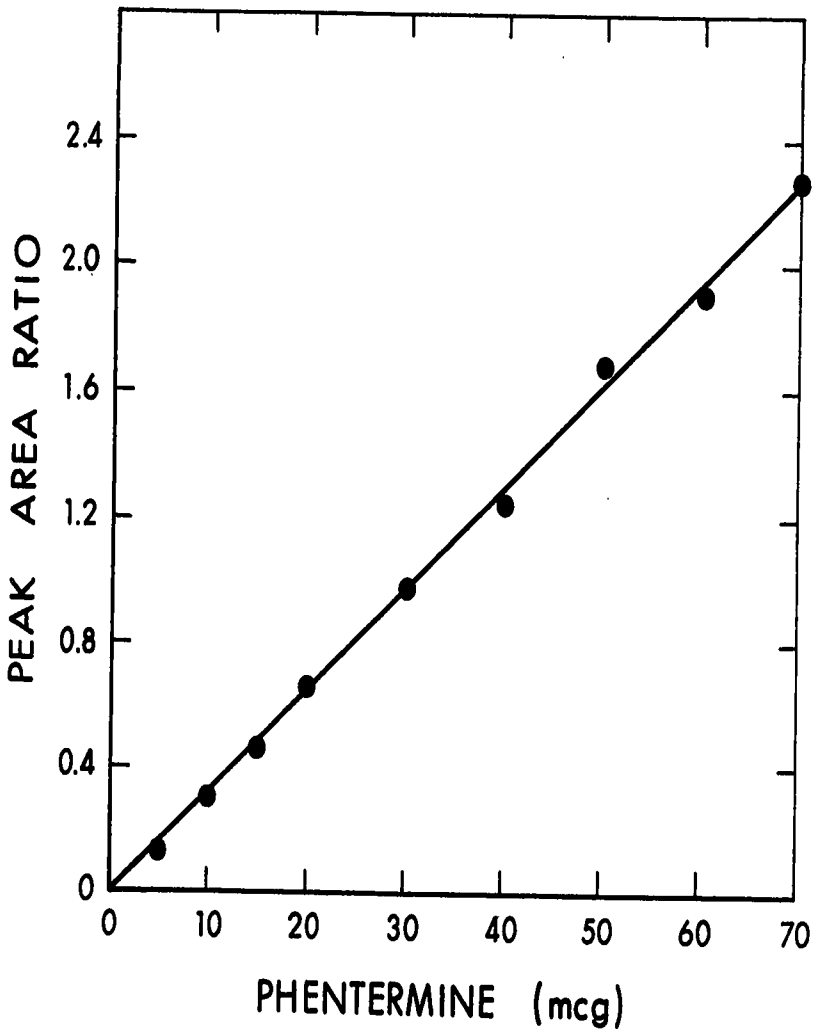

Figure 19: Calibration Curve for Phentermine in Distilled Water Using Hewlett-Packard Gas Chromatograph

Table XIII

Dissolution of Formulation I
Using U.S.P. Disintegration Apparatus

Dissolution Medium: Distilled Water
Volume: 900 ml

Time (Min)	Phentermine Dissolved (mcg)			% Dissolved
	Run 1	Run 2	Mean	
1	352	586	469.0	4.7
2	3146	3244	3195.0	32.2
5	6448	5946	6197.0	62.5
8	8140	7808	7944.0	80.4
12	8944	9043	8993.5	90.7
15	9175	9648	9411.5	94.9
20	9849	9869	9859.0	99.4
25	9848	9991	9919.5	100.0
30	9945	9732	9838.5	99.2
60	9839	9987	9913.0	100.0

Table XIV

Dissolution of Formulation II
Using U.S.P. Disintegration Apparatus

Dissolution Medium: Distilled Water
Volume: 900 ml

Time (Min)	Phentermine Dissolved (mcg)			% Dissolved
	Run 1	Run 2	Mean	
1	832	946	889.0	8.9
2	4232	4493	4362.5	43.7
5	7144	7539	7341.5	73.6
8	8693	8735	8714.0	87.4
12	9539	9732	9635.5	96.7
15	9789	9994	9891.5	99.2
20	9824	10058	9941.0	99.7
25	9885	9956	9920.5	99.5
30	9936	9999	9967.5	100.0
60	9951	9972	9961.5	100.0

Table XV

Dissolution of Formulation III
Using U.S.P. Disintegration Apparatus

Dissolution Medium: Distilled Water
Volume: 900 ml

Time (Min)	Phentermine Dissolved (mcg)			% Dissolved
	Run 1	Run 2	Mean	
1	459	398	428.5	4.3
2	1439	1173	1306.0	13.1
5	4559	4436	4497.5	45.2
8	7535	7494	7516.0	75.5
12	8838	8486	8662.0	87.0
15	9469	9287	9378.0	94.2
20	9549	9769	9659.0	97.0
25	9931	9849	9890.0	99.4
30	9836	10079	9957.5	100.0
60	9971	9925	9948.0	100.0

Table XVI

Dissolution of Formulation IV
Using U.S.P. Disintegration Apparatus

Dissolution Medium: Distilled Water
Volume: 900 ml

Time (Min)	Phentermine Dissolved (mcg)			
	Run 1	Run 2	Mean	% Dissolved
1	359	483	421.0	4.2
2	935	1086	1010.5	10.1
5	2936	2754	2845.0	28.6
8	5262	5639	5450.5	54.9
12	8736	8440	8588.0	86.6
15	9486	9115	9300.5	93.8
20	9655	9481	9568.0	96.5
25	9831	9645	9738.0	98.2
30	9886	9985	9935.5	100.2
60	9881	9948	9914.5	100.0

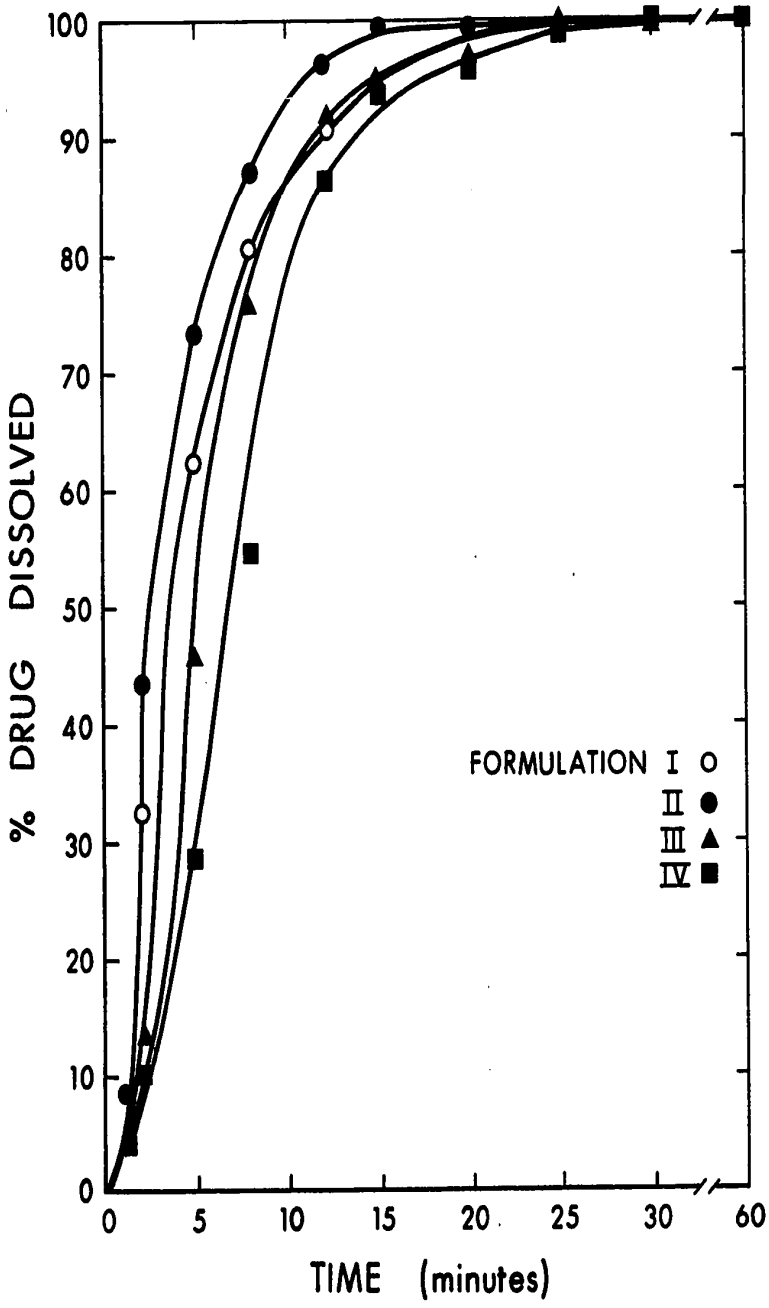

Figure 20: Dissolution Curves for Phentermine Tablet Formulations in Distilled Water Using U.S.P. Disintegration Apparatus

Table XVII

Dissolution of Formulation I
Using Rotating Basket Apparatus

Dissolution Medium: Distilled Water
Volume: 900 ml

Time (Min)	Phentermine Dissolved (mcg)			
	Run 1	Run 2	Mean	% Dissolved
5	606	598	602.0	6.1
10	4805	4536	4670.5	47.3
15	6781	6193	6487.0	65.7
20	6803	6853	6828.0	69.2
30	7515	8652	8083.5	81.9
40	6993	8532	7762.5	78.6
50	6971	8649	7810.0	79.1
60	7366	8832	8099.0	82.1
90	7585	8939	8262.0	83.7
∞	9880	9849	9864.5	100.0

Table XVIII

Dissolution of Formulation II
Using Rotating Basket Apparatus

Dissolution Medium: Distilled Water
Volume: 900 ml

Time (Min)	Phentermine Dissolved (mcg)			
	Run 1	Run 2	Mean	% Dissolved
5	2396	2939	2667.5	27.2
10	5592	5998	5795.0	59.2
15	5955	6732	6343.5	64.8
20	7132	7499	7315.5	74.9
30	8149	8936	8542.5	87.2
40	8436	8848	8642.0	88.3
50	8639	8748	8693.5	88.8
60	8758	8939	8848.5	90.4
90	8559	9136	8847.5	90.4
∞	9736	9835	9785.5	100.0

Table XIX

Dissolution of Formulation III
Using Rotating Basket Apparatus

Dissolution Medium: Distilled Water
Volume: 900 ml

Time (Min)	Phentermine Dissolved (mcg)			
	Run 1	Run 2	Mean	% Dissolved
5	450	432	441.0	4.5
10	2859	2639	2749.0	28.0
15	4228	4045	4136.5	42.2
20	5663	5832	5747.5	58.6
30	7634	7336	7485.0	76.4
40	8971	8739	8855.0	90.4
50	8720	8458	8589.0	87.7
60	8615	8913	8764.0	89.5
90	8943	9148	9045.5	92.3
∞	9738	9846	9792.0	100.0

Table XX

Dissolution of Formulation IV
Using Rotating Basket Apparatus

Dissolution Medium: Distilled Water
Volume: 900 ml

Time (Min)	Phentermine Dissolved (mcg)			
	Run 1	Run 2	Mean	% Dissolved
5	1127	987	1057.0	10.8
10	2205	2394	2299.5	23.6
15	3408	3248	3328.0	34.2
20	4576	4798	4687.0	48.2
30	6301	6839	6570.0	67.6
40	8261	8139	8200.0	84.3
50	8100	8666	8383.0	86.2
60	8490	8788	8639.0	88.8
90	8601	8936	8770.0	90.2
∞	9687	9749	9718.0	100.0

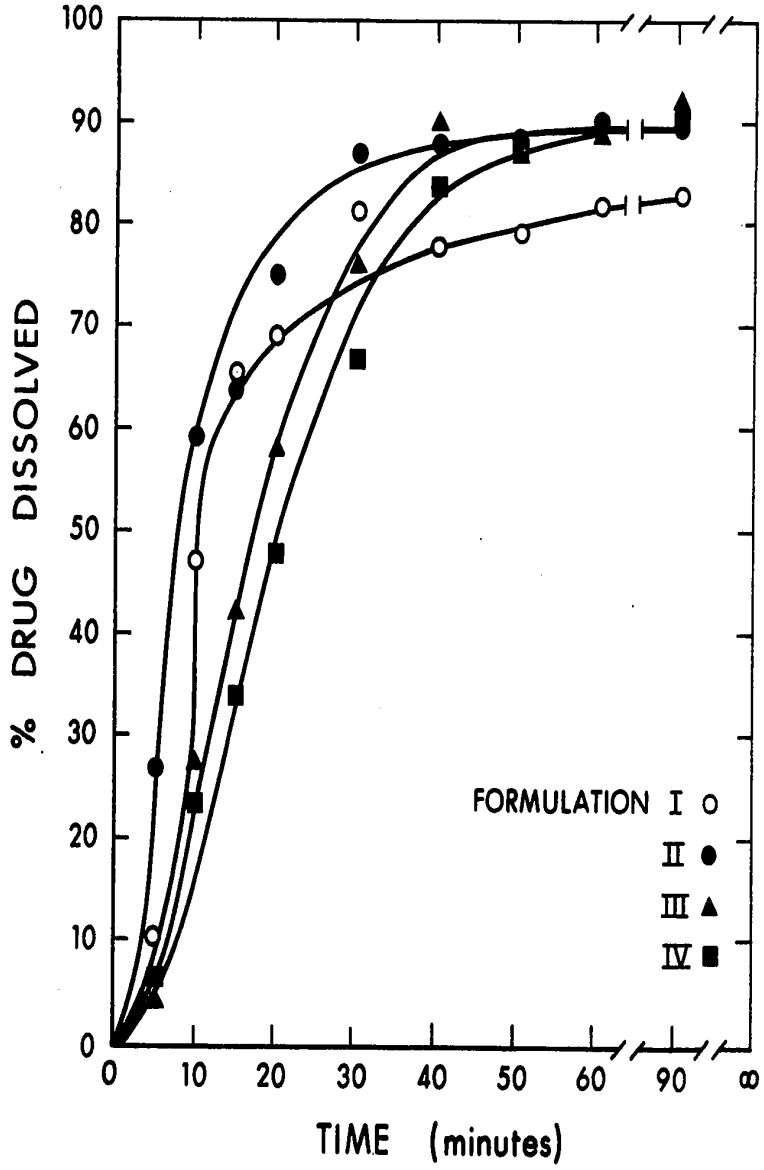

Figure 21: Dissolution Curves for Phentermine Tablet Formulations in Distilled Water Using Rotating Basket Apparatus

formulation are presented in Figure 21.

Dissolution Studies Using the Rotating Flask Apparatus

The dissolution studies were performed for each tablet formulation using the rotating flask method. Distilled water was used as the dissolution medium. The data for each tablet formulation are presented in Tables XXI - XXIV, and percent dissolved-time plots are presented in Figure 22.

On the basis of dissolution results obtained from three different dissolution apparatuses, it can be easily seen that the rotating flask apparatus is the best to distinguish the differences in tablet formulations under study. On the basis of this, further dissolution studies were conducted using the rotating flask apparatus.

Tables XXV - XXVIII contain dissolution data for each tablet formulation using simulated gastric fluid as the dissolution medium. Figure 23 shows the percent dissolved-time plot for each formulation. Percent undissolved-time plot is presented in Figure 24. Figure 25 shows a plot of percent dissolved on the probability scale against time on log scale according to the method described by Wagner (97).

Similar determinations were carried out using simulated intestinal fluid for each tablet formulation. The experiment was repeated for the Formulation II using pH 1.2

Table XXI

Dissolution of Formulation I
Using Rotating Flask Apparatus

Dissolution Medium: Distilled Water
Volume: 600 ml

Time (Min)	Phentermine Dissolved (mcg)			
	Run 1	Run 2	Mean	% Dissolved
5	453	423	438.0	4.4
10	1675	1450	1573.5	15.9
15	3439	3608	3523.5	35.7
20	4265	3949	4107.0	41.6
25	5614	5284	5449.0	55.2
30	6666	6498	6582.0	66.7
40	8348	8294	8321.0	84.3
50	8855	8346	8600.5	87.1
60	9015	9428	9221.5	93.4
90	9826	9629	9727.5	98.6
∞	9998	9729	9863.5	100.0

Table XXII

Dissolution of Formulation II
Using Rotating Flask Apparatus

Dissolution Medium: Distilled Water
Volume: 600 ml

Time (Min)	Phentermine Dissolved (mcg)			% Dissolved
	Run 1	Run 2	Mean	
5	958	958	958.0	9.7
10	3778	3802	3790.0	38.4
15	6105	6259	6182.0	62.6
20	6939	7128	7033.5	71.2
25	7748	7659	7703.5	78.0
30	8394	8596	8495.0	86.0
40	8750	8852	8801.0	89.1
50	9518	9597	9557.5	96.8
60	9734	9984	9859.0	99.9
90	9809	9915	9862.0	99.9
∞	9850	9886	9868.0	100.0

Table XXIII

Dissolution of Formulation III
Using Rotating Flask Apparatus

Dissolution Medium: Distilled Water
Volume: 600 ml

Time (Min)	Phentermine Dissolved (mcg)			% Dissolved
	Run 1	Run 2	Mean	
5	254	192	223.0	2.3
10	958	839	898.5	9.2
15	2211	2148	2179.5	22.4
20	2800	2672	2736.0	28.1
25	4255	4621	4438.0	45.7
30	4836	5439	5137.5	52.9
40	7241	7439	7340.0	75.6
50	8565	8433	8499	87.5
60	9372	9628	9500	97.8
90	9586	9879	9732.5	100.2
∞	9479	9936	9707.5	100.0

Table XXIV

Dissolution of Formulation IV
Using Rotating Flask Apparatus

Dissolution Medium: Distilled Water
Volume: 600 ml

Time (Min)	Phentermine Dissolved (mcg)			% Dissolved
	Run 1	Run 2	Mean	
5	-	189	189.0	1.9
10	458	439	448.5	4.5
15	979	647	813.0	8.2
20	2104	1865	1984.5	20.0
25	3364	2954	3159.0	31.8
30	4149	4064	4106.5	41.4
40	5546	5409	5477.5	55.2
50	7186	7725	7455.5	75.2
60	9136	8954	9045.0	91.3
90	9699	9845	9772.0	98.6
∞	10052	9759	9905.5	100.0

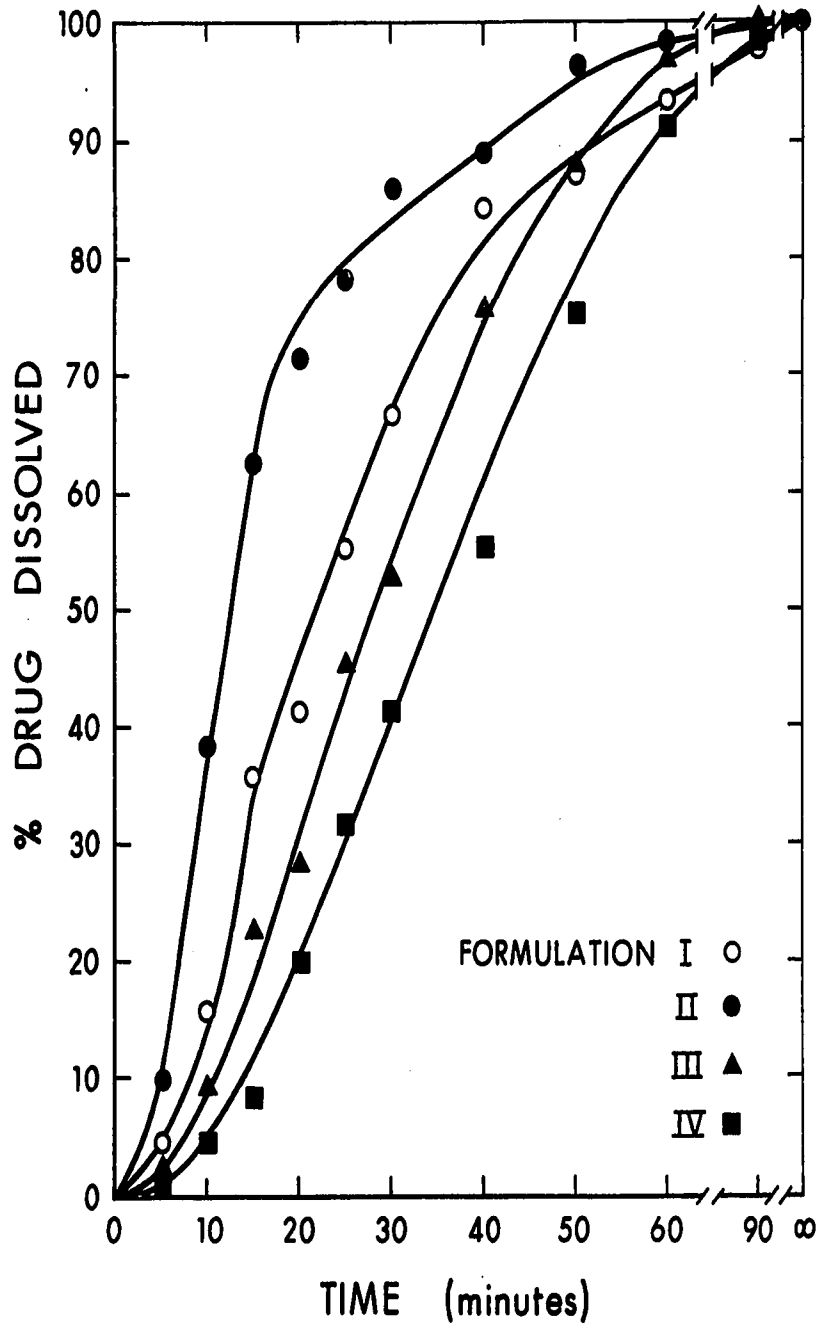

Figure 22: Dissolution Curves for Phentermine Tablet Formulations in Distilled Water Using Rotating Flask Apparatus

Table XXV

Dissolution of Formulation I
Using Rotating Flask Apparatus

Dissolution Medium: Simulated Gastric Fluid, U.S.P.
Volume: 600 ml

Time (Min)	Phentermine Dissolved (mcg)			% Dissolved	% Undissolved
	Run 1	Run 2	Mean		
5	350.36	358.84	354.60	3.6	96.4
10	1390.21	1407.19	1398.70	14.2	85.8
15	2330.36	2358.24	2344.30	23.8	76.2
20	4273.61	4295.89	4284.75	43.5	56.5
30	6551.36	6588.54	6569.95	66.7	33.3
40	7836.31	7805.49	7820.90	79.4	20.6
50	8630.14	8607.36	8618.75	87.5	12.5
60	9119.72	9102.78	9111.25	92.5	7.5
90	9655.39	9651.21	9653.30	98.0	2.0
∞	9860.31	9839.81	9850.06	100.0	0.0

Table XXVI

Dissolution of Formulation II
Using Rotating Flask Apparatus

Dissolution Medium: Simulated Gastric Fluid, U.S.P.
Volume: 600 ml

Time (Min)	Phentermine Dissolved (mcg)			% Dissolved	% Undissolved
	Run 1	Run 2	Mean		
5	920.39	932.17	926.28	9.3	90.7
10	3700.14	3749.94	3725.04	37.4	62.6
15	5761.36	5792.24	5776.80	58.0	42.0
20	6931.12	6953.12	6942.12	69.7	30.3
30	8370.21	8422.35	8396.28	84.3	15.7
40	9242.19	9203.73	9222.96	92.6	7.4
50	9621.08	9581.80	9601.44	96.4	3.6
60	9720.13	9801.47	9760.47	98.0	2.0
90	9946.49	9929.75	9938.12	99.8	0.2
∞	9936.38	9983.62	9960.00	100.0	0.0

Table XXVII

Dissolution of Formulation III
Using Rotating Flask Apparatus

Dissolution Medium: Simulated Gastric Fluid, U.S.P.
Volume: 600 ml

Time (Min)	Phentermine Dissolved (mcg)			% Dissolved	% Undissolved
	Run 1	Run 2	Mean		
5	118.36	161.36	139.86	1.4	98.6
10	988.44	949.62	969.03	9.7	90.3
15	2301.13	2234.33	2267.73	22.7	77.3
20	3571.25	3541.63	3556.44	35.6	64.4
30	5532.44	5476.54	5504.49	55.1	44.9
40	6989.36	6936.70	6936.03	69.7	30.3
50	7956.01	7908.11	7932.06	79.4	20.6
60	8549.39	8533.51	8541.45	85.5	14.5
90	9539.36	9481.60	9510.48	95.2	4.8
∞	10020.10	9959.90	9990.00	100.0	0.0

Table XXVIII

Dissolution of Formulation IV
Using Rotating Flask Apparatus

Dissolution Medium: Simulated Gastric Fluid, U.S.P.
Volume: 600 ml

Time (Min)	Phentermine Dissolved (mcg)			% Dissolved	% Undissolved
	Run 1	Run 2	Mean		
5	135.39	81.79	108.59	1.1	98.9
10	367.51	323.53	345.52	3.5	96.5
15	1401.36	1343.04	1372.20	13.9	86.1
20	2461.31	2395.71	2428.51	24.6	75.4
30	4051.39	3984.41	4017.90	40.7	59.3
40	5431.82	5348.40	5390.11	54.6	45.4
50	6413.19	6361.17	6387.18	64.7	35.3
60	7825.11	7831.87	7828.49	79.3	20.7
90	8462.36	8399.00	8430.68	85.4	14.6
∞	9909.36	9834.66	9872.01	100.0	0.0

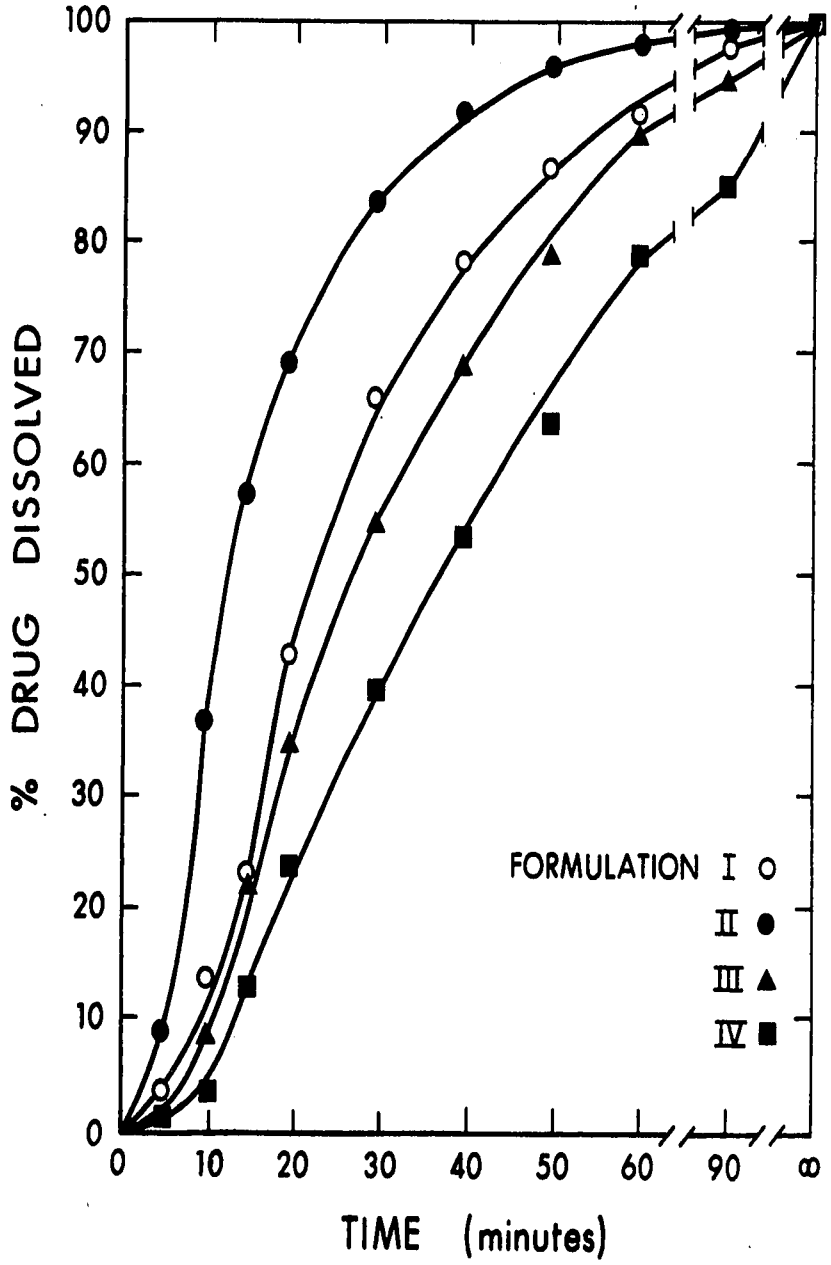

Figure 23: Dissolution Curves for Phentermine Tablet Formulations in Simulated Gastric Fluid Using Rotating Flask Apparatus

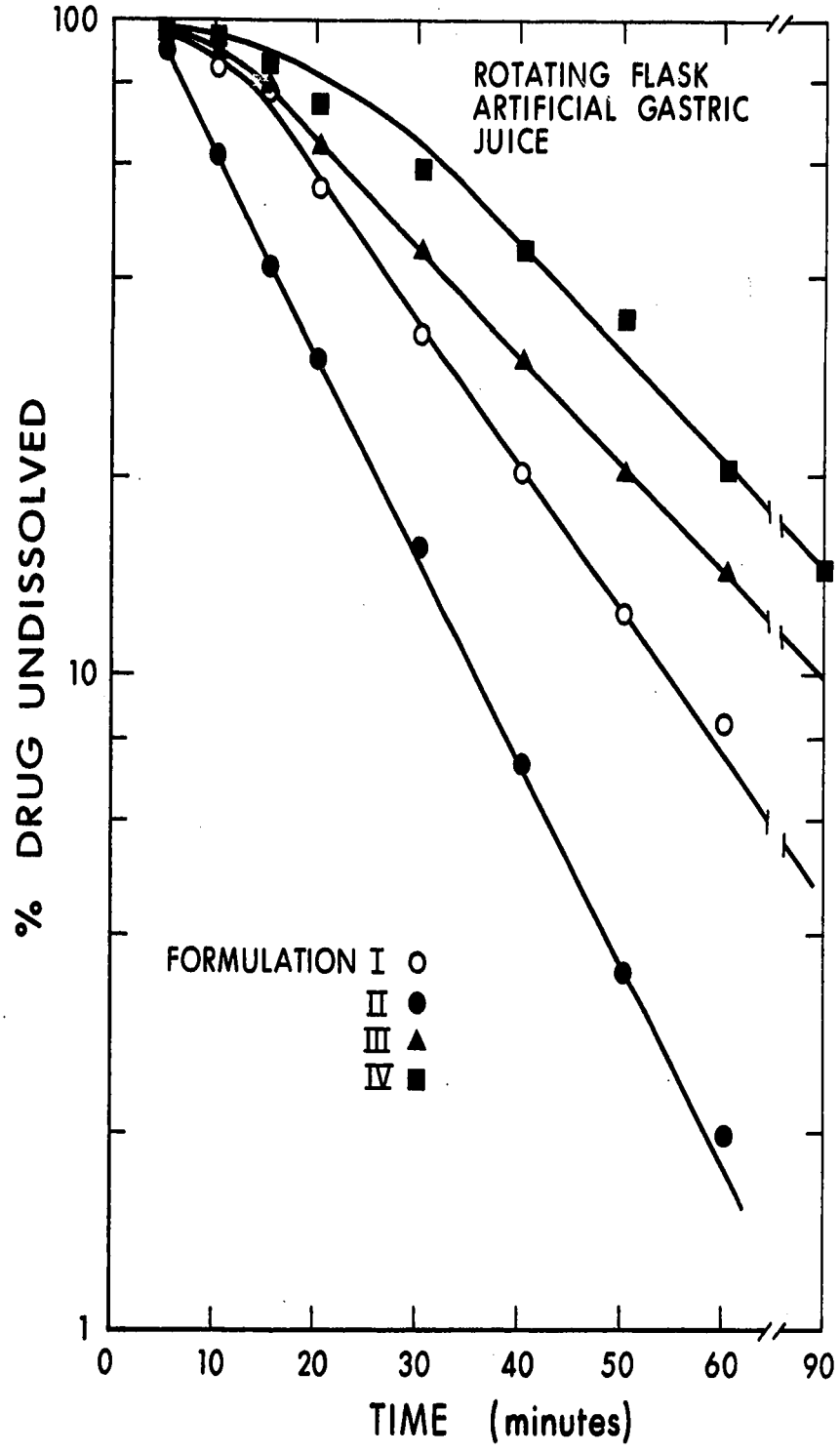

Figure 24: Semilogarithmic Plot of Percent Phentermine Undissolved from Tablet Formulations Against Time in Simulated Gastric Fluid Using Rotating Flask Apparatus

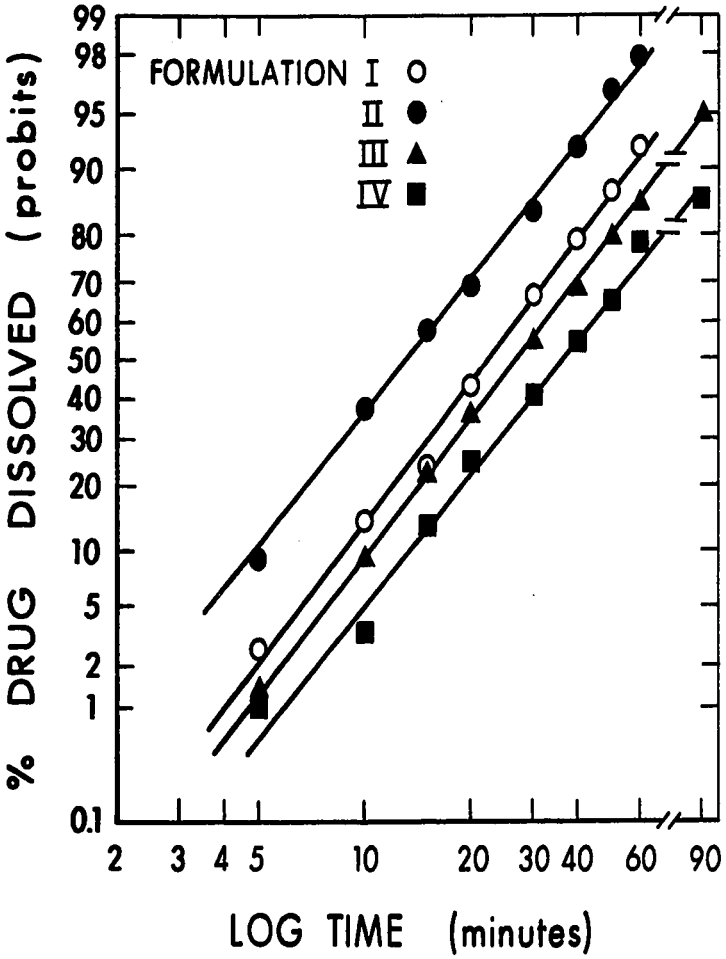

Figure 25: Log-Normal Probability Plots of Dissolution Data from Phentermine Tablet Formulations in Simulated Gastric Fluid

hydrochloric acid solution and pH 7.5 phosphate buffer. The data for these studies are presented in Tables XXIX - XXXIV and the results are presented graphically in Figures 26 - 34.

pH CHANGES IN VARIOUS DISSOLUTION MEDIA

The changes in pH in various dissolution media due to the dissolution of phentermine tablet Formulation II are presented in Table XXXV.

BINDING STUDIES

The evidence of binding between phentermine and the tablet diluents was investigated using the equilibrium dialysis method. The results are presented in Table XXXVI.

BIOAVAILABILITY OF PHENTERMINE TABLET FORMULATIONS

The relative bioavailability of all the tablet formulations of phentermine was calculated from the cumulative amount of the unchanged drug excreted in the urine in 54 hours. The following equation was used to calculate relative percent bioavailability.

$$\text{relative percent bioavailability} = \frac{\left(\begin{array}{c}\%\text{ of dose recovered} \\ \text{in urine at time } t\end{array}\right)_{\text{test preparation}}}{\left(\begin{array}{c}\%\text{ of dose recovered} \\ \text{in urine in time } t\end{array}\right)_{\text{(solution)}}}$$

(Equation 16)

The results are presented in Table XXXVII.

Table XXIX

Dissolution of Formulation I
Using Rotating Flask Apparatus

Dissolution Medium: Simulated Intestinal Fluid, U.S.P.
Volume: 600 ml

Time (Min)	Phentermine Dissolved (mcg)			% Dissolved	% Undissolved
	Run 1	Run 2	Mean		
5	305.61	355.97	330.79	3.3	96.7
10	1251.35	1274.69	1263.02	12.6	87.4
15	2631.35	2681.37	2656.36	26.5	73.5
20	4319.46	4381.38	4350.42	43.4	56.6
30	6498.91	6552.33	6525.62	65.1	34.9
40	7762.45	7794.79	7778.62	77.6	22.4
50	8672.16	8669.36	8670.76	86.5	13.5
60	9250.12	9274.24	9262.18	92.4	7.6
90	9534.48	9571.26	9552.87	95.3	4.7
∞	9996.39	10051.61	10024.00	100.0	0.0

Table XXX

Dissolution of Formulation II
Using Rotating Flask Apparatus

Dissolution Medium: Simulated Intestinal Fluid, U.S.P.
Volume: 600 ml

Time (Min)	Phentermine Dissolved (mcg)			% Dissolved	% Undissolved
	Run 1	Run 2	Mean		
5	830.13	805.37	817.75	8.2	91.8
10	2979.35	2964.29	2971.35	29.8	70.2
15	5499.10	5470.72	5484.91	55.0	45.0
20	6661.19	6622.25	6641.72	66.6	33.4
30	8211.35	8183.53	8179.44	82.2	17.8
40	9169.15	9140.47	9154.81	91.8	8.2
50	9640.41	9606.63	9623.52	96.5	3.5
60	9790.18	9795.92	9793.05	98.2	1.8
90	9960.75	9980.37	9970.56	100.0	0.0
∞	9998.73	9946.39	9972.56	100.0	0.0

Table XXXI

Dissolution of Formulation III
Using Rotating Flask Apparatus

Dissolution Medium: Simulated Intestinal Fluid, U.S.P.
Volume: 600 ml

Time (Min)	Phentermine Dissolved (mcg)			% Dissolved	% Undissolved
	Run 1	Run 2	Mean		
5	169.16	128.20	148.68	1.5	98.5
10	880.92	863.66	872.29	8.8	91.2
15	1654.66	1616.42	1635.54	16.5	83.5
20	3156.39	3128.05	3142.22	31.7	68.3
30	5147.66	5121.54	5134.60	51.8	48.2
40	6950.46	6946.66	6948.56	70.1	29.9
50	7879.19	7861.63	7870.41	79.4	20.6
60	8430.66	8380.70	8405.68	84.4	15.2
90	9044.37	9015.95	9030.16	91.1	8.9
∞	9936.11	9888.61	9912.36	100.0	0.0

Table XXXII

Dissolution of Formulation IV
Using Rotating Flask Apparatus

Dissolution Medium: Simulated Intestinal Fluid, U.S.P.
Volume: 600 ml

Time (Min)	Phentermine Dissolved (mcg)			% Dissolved	% Undissolved
	Run 1	Run 2	Mean		
5	111.35	127.89	119.62	1.2	98.8
10	261.56	296.68	279.12	2.8	97.2
15	775.69	799.35	787.52	7.9	92.1
20	1231.96	1240.26	1236.11	12.4	87.6
30	3063.45	3077.25	2070.35	30.8	69.2
40	5546.39	5558.71	5552.55	55.7	44.3
50	6408.92	6430.72	6419.82	64.4	35.6
60	7830.36	7820.46	7825.41	78.5	21.5
90	8539.42	8566.96	8553.19	85.8	14.2
∞	9960.46	9976.88	9968.67	100.0	0.0

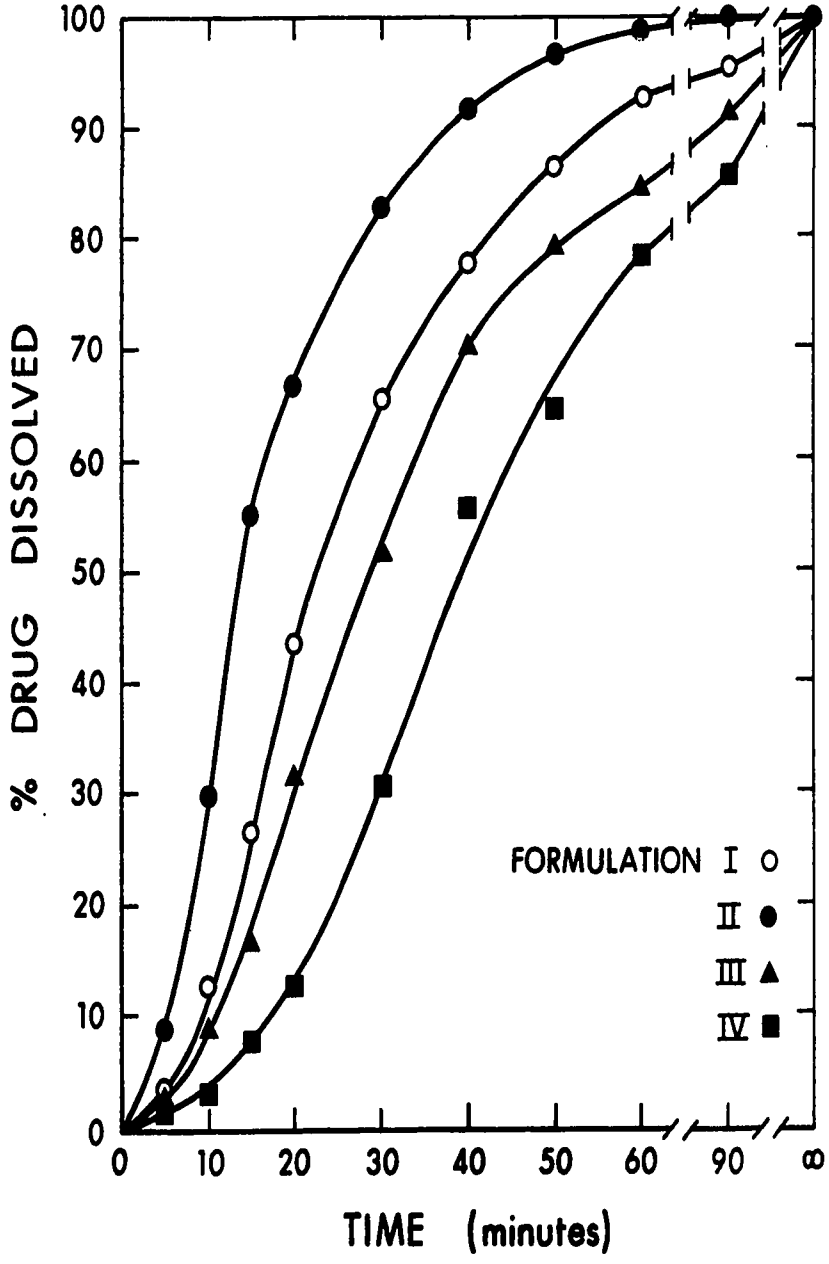

Figure 26: Dissolution Curves for Phentermine Tablet Formulations in Simulated Intestinal Fluid Using Rotating Flask Apparatus

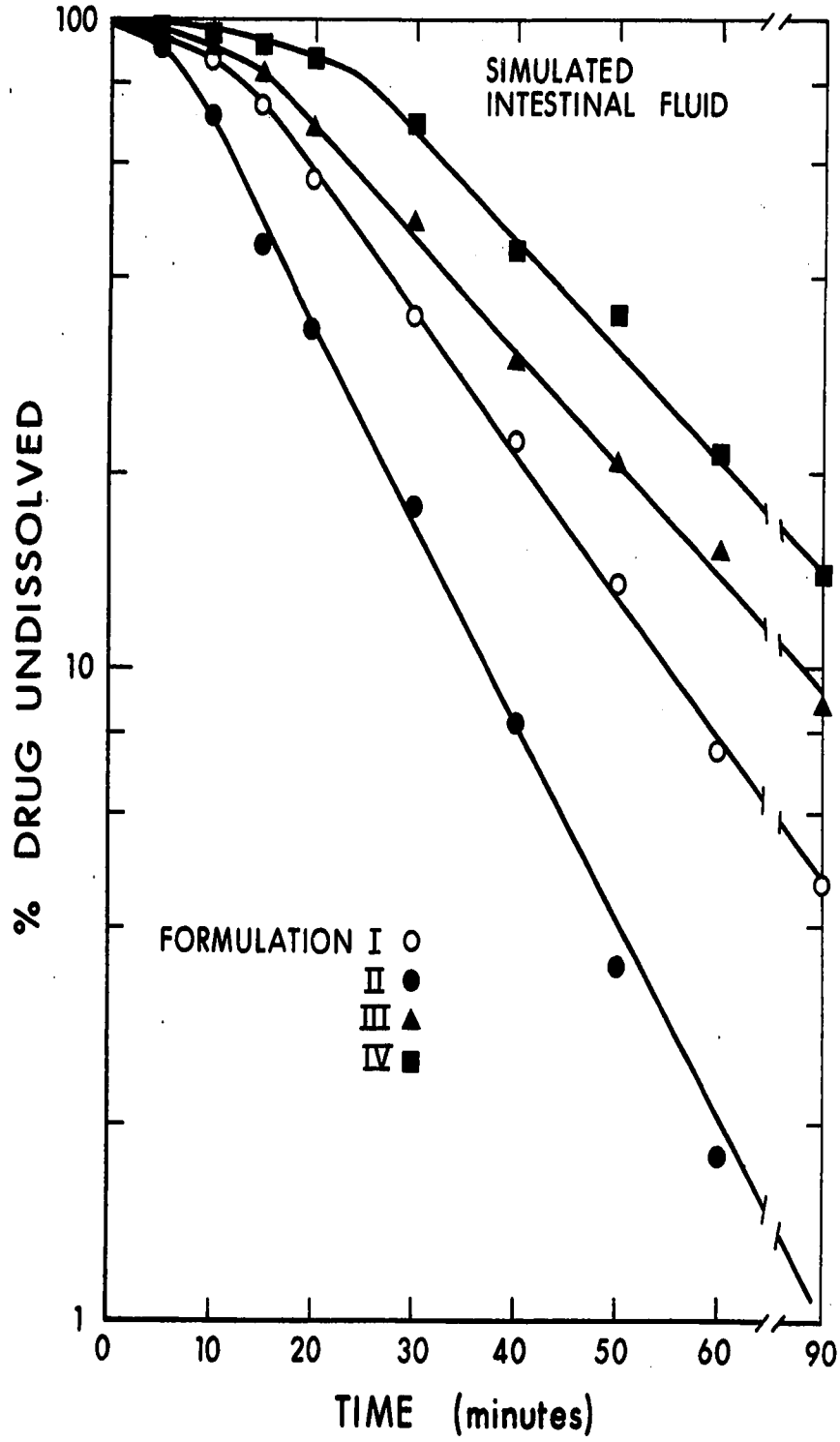

Figure 27: Semilogarithmic Plot of Percent Phentermine Undissolved from Tablet Formulations Against Time in Simulated Intestinal Fluid Using Rotating Flask Apparatus

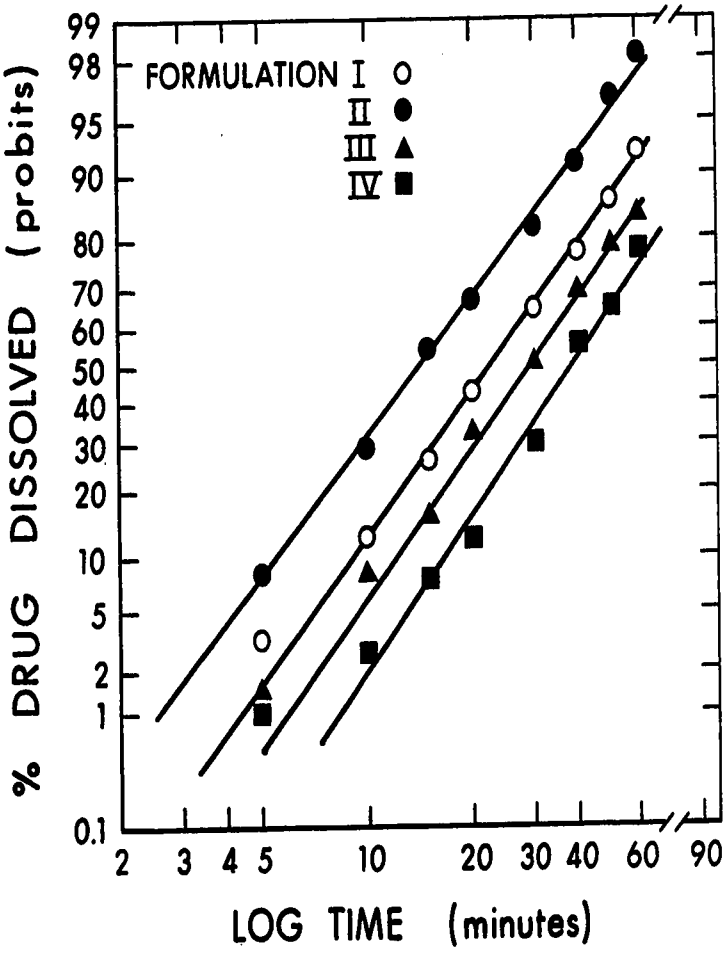

Figure 28: Log-Normal Probability Plots of Dissolution Data from Phentermine Tablet Formulations in Simulated Intestinal Fluid

Table XXXIII

Dissolution of Formulation II
Using Rotating Flask Apparatus

Dissolution Medium: Hydrochloric Acid Solution, pH 1.2
Volume: 600 ml

Time (Min)	Phentermine Dissolved (mcg)			% Dissolved	% Undissolved
	Run 1	Run 2	Mean		
5	976.49	1228.36	1102.42	11.1	88.9
10	3403.22	4115.03	3759.12	37.8	62.2
15	5829.94	5742.63	5786.28	58.1	41.9
20	6825.77	6858.39	6842.08	68.7	31.3
30	8256.66	8864.73	8560.69	86.0	14.0
40	9030.13	9468.67	9249.40	92.9	7.1
50	9358.85	9878.13	9618.49	96.6	3.4
60	9474.86	10052.15	9763.51	98.1	1.9
90	9658.56	10186.36	9922.49	99.7	0.3
∞	9668.23	10236.41	9952.32	100.0	0.0

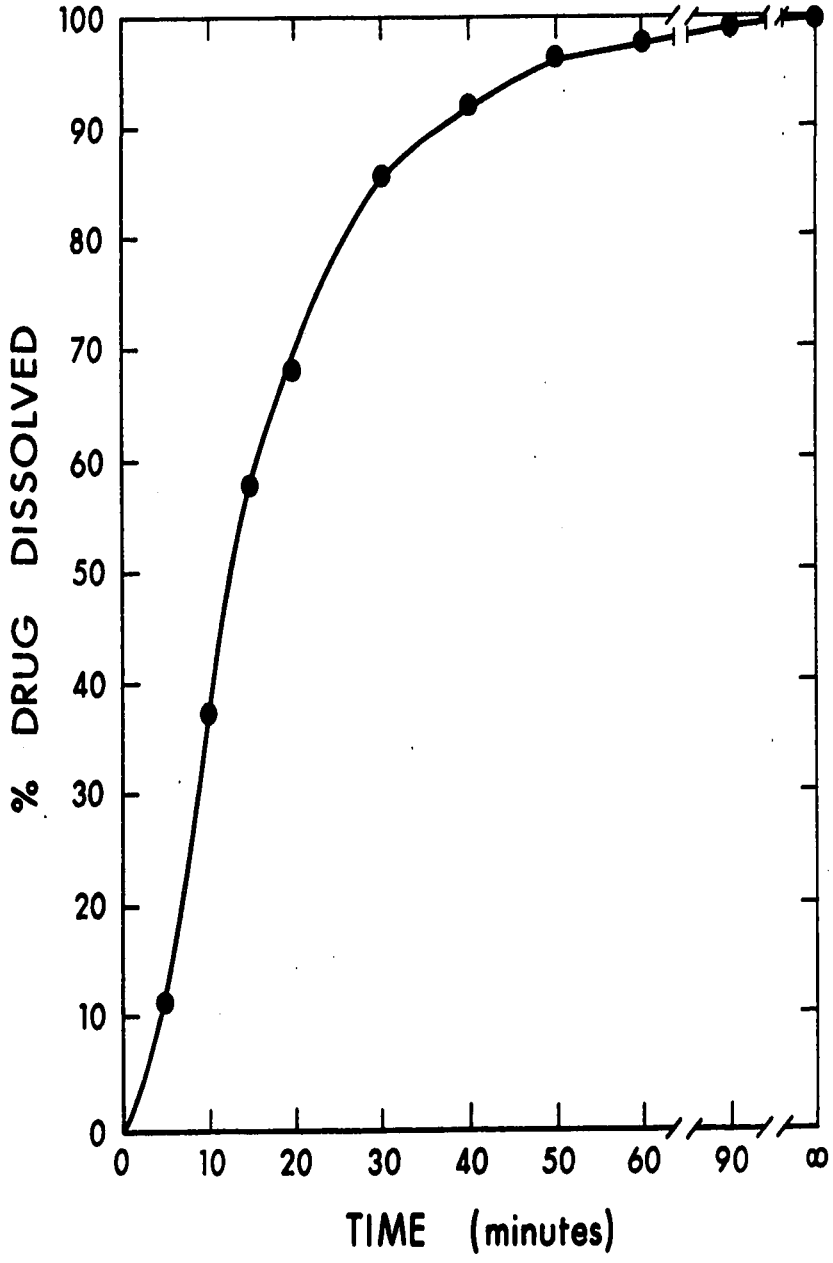

Figure 29: Dissolution Curve for Phentermine Tablet Formulation II in Dilute Hydrochloric Acid (pH 1.2)

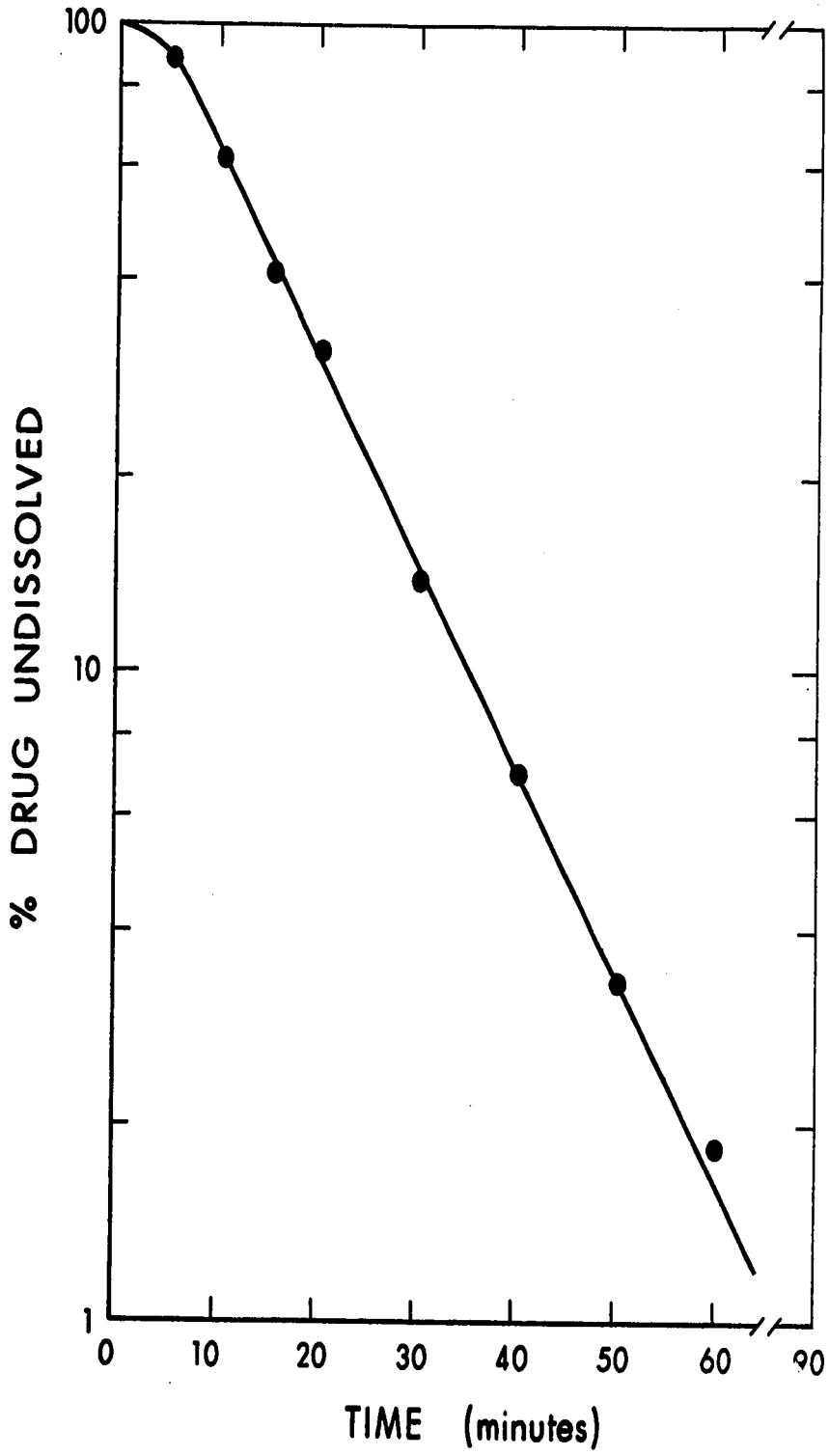

Figure 30: Semilogarithmic Plot of Percent Phentermine Undissolved from Formulation II in Dilute Hydrochloric Acid Using Rotating Flask Apparatus

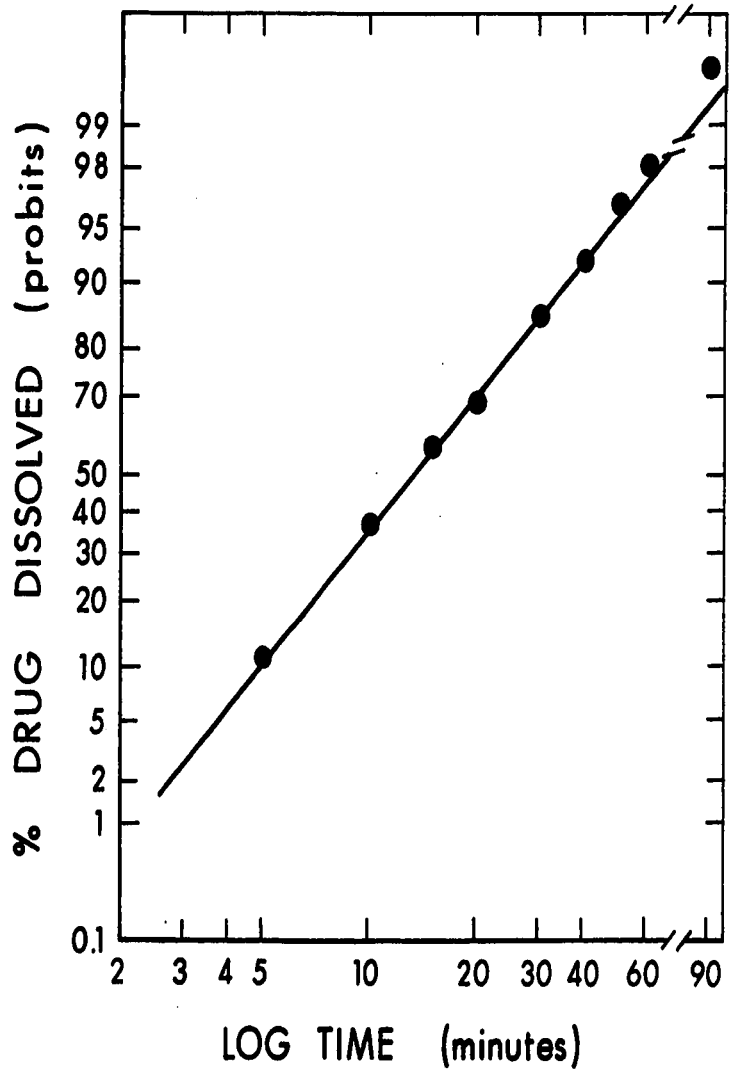

Figure 31: Log-Normal Probability Plot of Dissolution Data from Phentermine Tablet Formulation II in Dilute Hydrochloric Acid

Table XXXIV

Dissolution of Formulation II
Using Rotating Flask Apparatus

Dissolution Medium: Phosphate Buffer, pH 7.5
Volume: 600 ml

Time (Min)	Phentermine Dissolved (mcg)			% Dissolved	% Undissolved
	Run 1	Run 2	Mean		
5	379.71	674.66	527.18	5.2	94.8
10	2515.23	2871.46	2693.35	26.8	73.2
15	5128.68	5057.76	5093.22	50.6	49.4
20	6539.20	6755.67	6647.43	66.1	33.9
30	8555.24	8450.58	8502.91	85.5	14.5
40	9395.67	9203.66	9299.66	92.5	7.5
50	9789.56	9687.06	9738.31	96.8	3.2
60	9999.17	9802.92	9901.05	98.5	1.5
90	10120.39	9865.96	9993.17	99.4	0.6
∞	10125.74	9987.69	10056.72	100.0	0.0

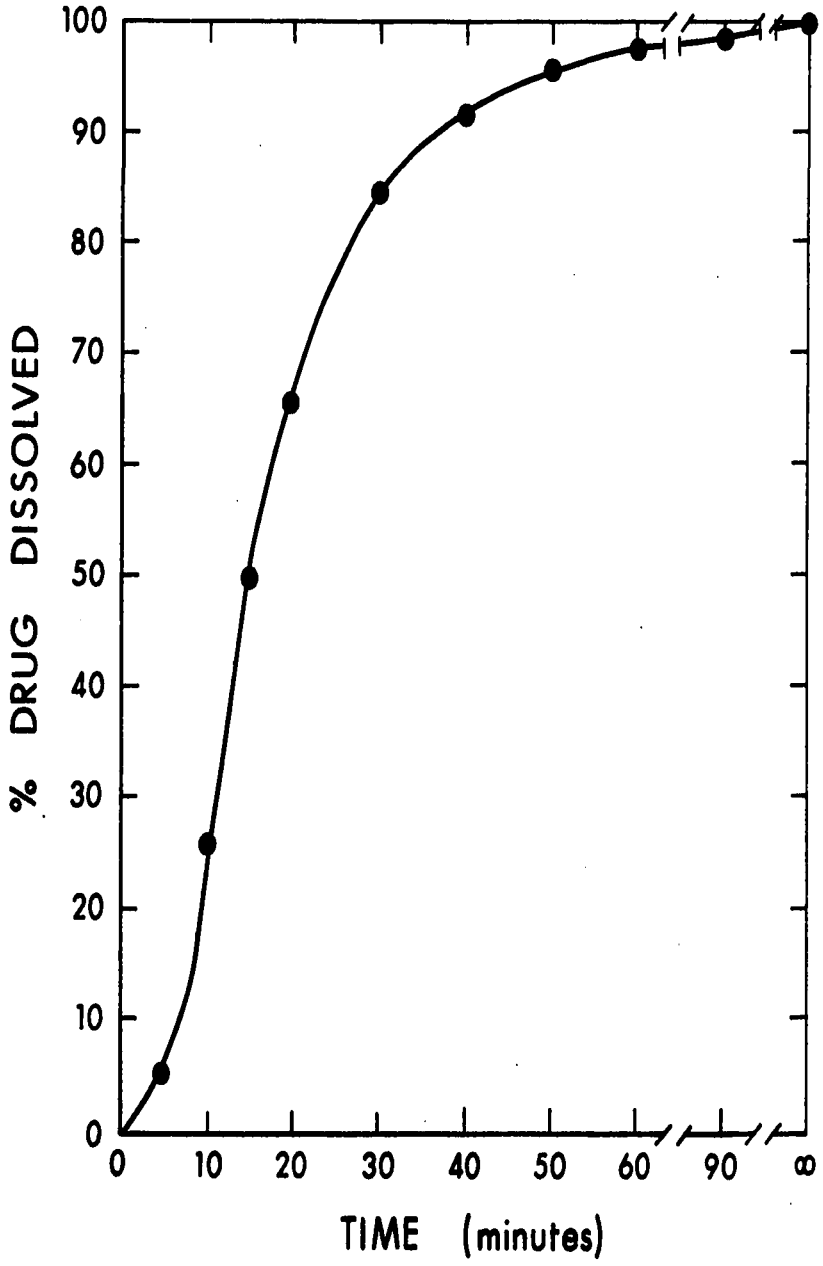

Figure 32: Dissolution Curve for Phentermine Tablet Formulation II in Phosphate Buffer Using Rotating Flask Apparatus

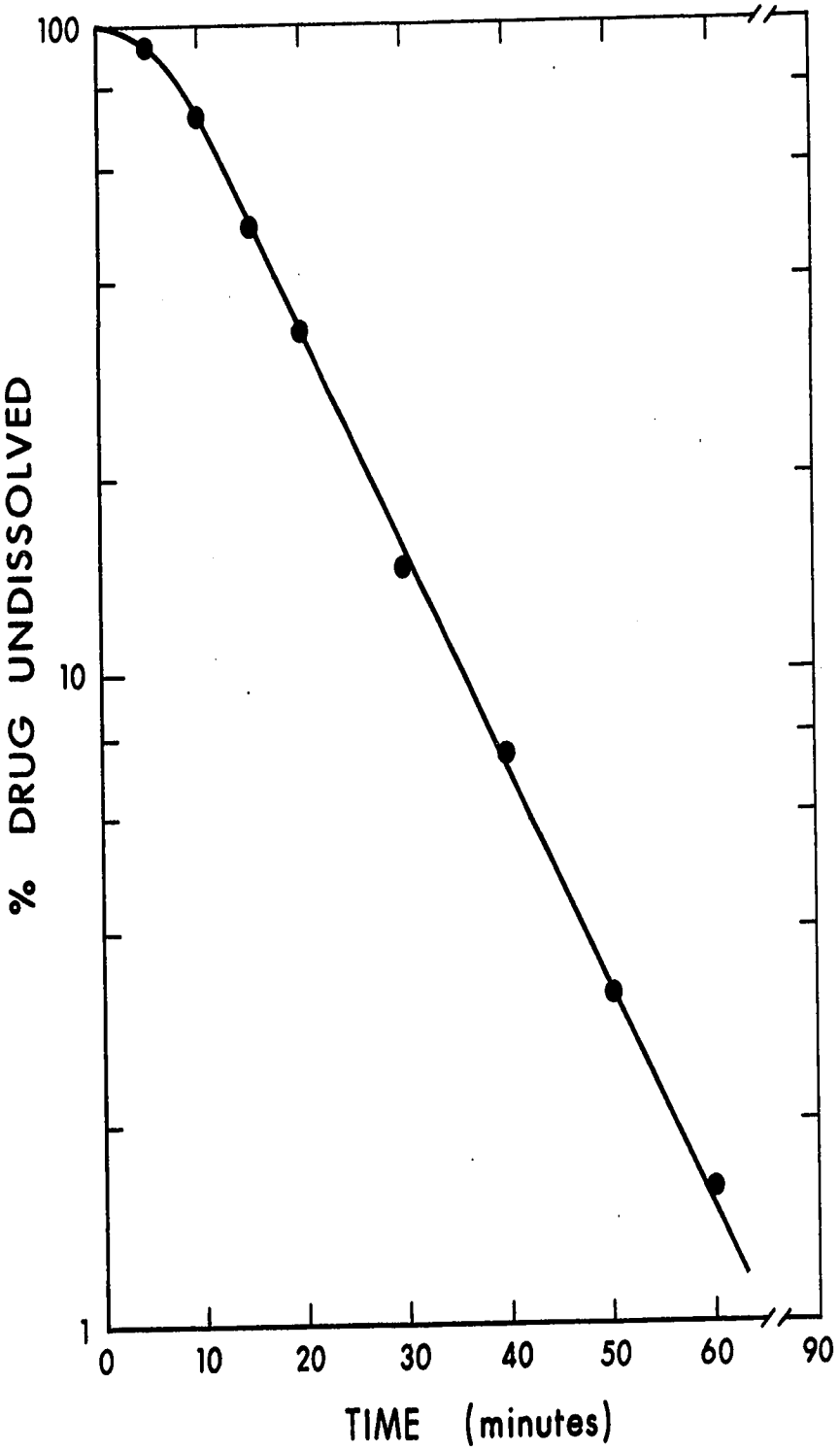

Figure 33: Semilogarithmic Plot of Percent Phentermine Undissolved from Tablet Formulation II Against Time in Phosphate Buffer Using Rotating Flask Apparatus

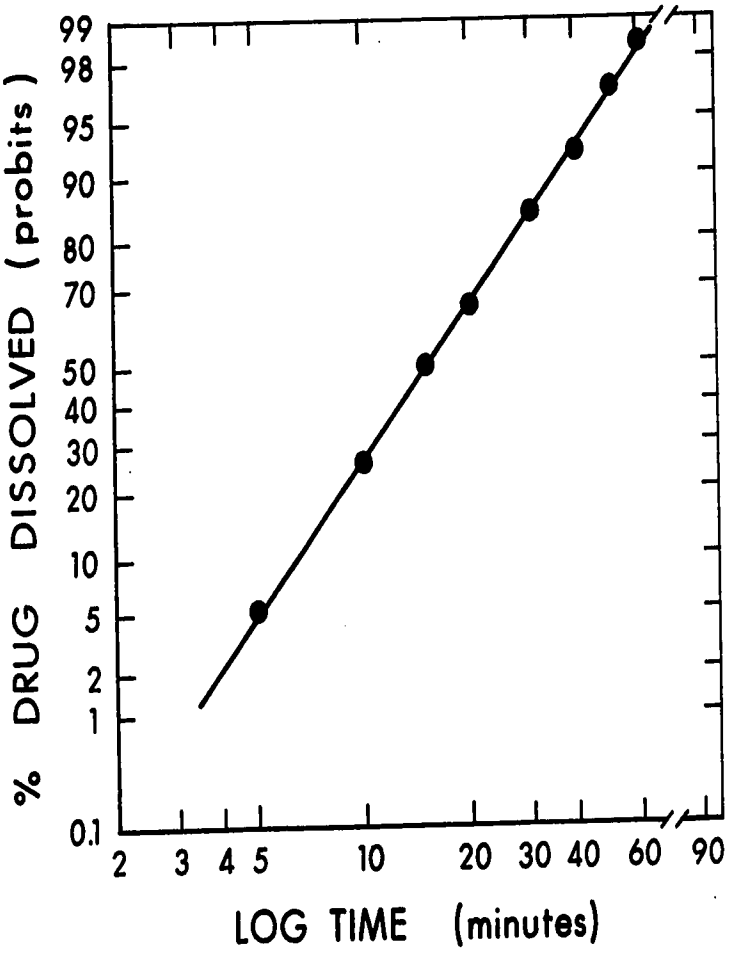

Figure 34: Log-Normal Probability Plot of Dissolution Data for Formulation II in Phosphate Buffer

Table XXXV

pH Changes in Various Dissolution Media in the Presence of Formulation II

Dissolution Media	pH of the Dissolution Media	pH After the Dissolution of Formulation II
Distilled Water	6.60	6.80
Hydrochloric Acid Solution	1.20	1.20
Unbuffered Solution	7.50	8.00
Phosphate Buffer	7.50	7.50
Simulated Gastric Fluid	1.20	1.20
Simulated Intestinal Fluid	7.50	7.65

Table XXXVI

Binding Studies Between Phentermine and Tablet Diluents

Amount of Phentermine on Side I Before Equilibrium: 10072.93 mcg

Diluent	Phentermine (mcg) on Side I[b]	Phentermine (mcg) on Side II[b]
Control	4991 ± 18.3	5007 ± 2.3
Lactose[a]	5027 ± 32.1	5032 ± 17.9
Dextrose[a]	4995 ± 30.1	4974 ± 46.3
Calcium Phosphate[a]	4982 ± 79.8	4989 ± 64.5
Calcium Phosphate (Saturated Solution)[c]	4941 ± 17.5	4939 ± 31.5

[a] 250 mg of the diluent on Sides I and II

[b] mean of three determinations with the standard error

[c] saturated solution of calcium phosphate and 10042.38 mcg of phentermine on Side I

Table XXXVII

Relative Percent Bioavailabilities of Phentermine Tablet Formulations in Various Subjects

Formulation No.	Relative % Bioavailability			
	Subject JM	Subject SM	Subject HC	Subject GP
I	86.16	84.65	86.08	100.00
II	98.16	97.51	110.30	106.47
III	76.08	83.88	71.74	90.93
IV	70.68	78.43	63.37	88.70

The analysis of variance using 4 X 4 latin square design (217) was used to test the significance of results obtained. A significant difference ($p < 0.005$) between the relative percent bioavailabilities from different formulations was obtained excepting formulations III and IV.

ANALOG AND DIGITAL COMPUTER SIMULATION

The Analog and Digital Computers were used to evaluate the pharmacokinetic parameters for phentermine and to confirm the validity of the chosen one compartmental model. A good fit between the experimental data and computer

The cumulative amounts of phentermine excreted

unchanged in the urine were plotted against time on a graph paper. The rate constant potentiometers were varied to obtain a good fit between the experimental data points and computer generated curves. The lag times were adjusted manually by moving the recorder pen. The rate constants thus obtained are presented in Table XXXVIII.

Using digital computer program 'Nonlin' (201) an attempt was made to fit the urinary excretion data with the computer generated curves. A poor fit was obtained first since the program does not allow compensation for the lag time. To overcome this problem, the lag times obtained from the analog computer were subtracted from the time of the urine collection, and this time scale was supplied to the computer along with the corresponding cumulative amounts of drug excreted in the urine. A good fit was obtained. The results are presented in Table XXXIX.

Table XXXVIII

Rate Constants and Biological Half-lives of Phentermine in Four Subjects Using Analog Computer

Formulation	$K_A(hr^{-1})$	$K_u(hr^{-1})$	$T_{1/2}$ Excretion (hr)	lag time (hr)
Solution (GP)	2.090	0.066	10.5	1.00
I (GP)	1.320	0.066	10.5	1.00
II (GP)	0.824	0.063	11.0	1.25
III (GP)	0.694	0.068	10.0	1.50
IV (GP)	0.650	0.066	10.5	1.25
Solution (JM)	0.890	0.077	9.0	1.00
I (JM)	0.560	0.081	8.5	1.75
II (JM)	0.550	0.099	7.0	1.50
III (JM)	0.450	0.092	7.5	1.75
IV (JM)	0.500	0.091	7.6	1.75
Solution (SM)	2.600	0.076	9.1	0.75
I (SM)	1.550	0.073	9.4	1.25
II (SM)	1.300	0.078	8.8	1.25
III (SM)	0.990	0.077	9.0	1.50
IV (SM)	0.960	0.075	9.2	1.50
Solution (HC)	5.210	0.069	10.0	0.50
I (HC)	1.350	0.072	9.6	1.00
II (HC)	1.000	0.077	9.0	1.50
III (HC)	1.110	0.072	9.6	1.50
IV (HC)	0.750	0.070	9.8	1.50

Table XXXIX

Rate Constants and Biological Half-lives of Phentermine in Four Subjects Using NONLIN

Formulation	$K_A(hr^{-1})$	$K_u(hr^{-1})$	$T_{1/2}$ Excretion (hr)
Solution (GP)	1.8034	0.0761	9.98
I (GP)	1.2248	0.0659	10.62
II (GP)	0.6185	0.0703	9.96
III (GP)	0.5386	0.0693	10.10
IV (GP)	0.6639	0.0702	9.97
Solution (JM)	0.8759	0.0753	9.29
I (JM)	0.6139	0.0795	8.81
II (JM)	0.5149	0.0976	7.17
III (JM)	0.5031	0.0938	7.46
IV (JM)	0.5314	0.0909	7.70
Solution (SM)	2.5504	0.0759	9.22
I (SM)	1.4565	0.0751	9.32
II (SM)	1.5392	0.0792	8.84
III (SM)	0.8350	0.0765	9.15
IV (SM)	0.8232	0.0748	9.36
Solution (HC)	4.4139	0.0691	10.13
I (HC)	1.3952	0.0700	10.00
II (HC)	1.2136	0.0715	9.79
III (HC)	1.2006	0.0723	9.68
IV (HC)	0.9130	0.0714	9.80

DISCUSSION

Four tablet formulations, each containing 10 mg phentermine, were prepared so that each formulation had different disintegration and dissolution characteristics. It was found that the use of different diluents and granulating agents gave good control over the disintegration and dissolution characteristics of the tablet formulations prepared. Thus calcium phosphate and 10% starch paste gave a fast disintegrating tablet and dextrose and starch and acacia mucilage gave a slowly disintegrating tablet. Combination of lactose with starch paste and dextrose with starch paste gave tablets with intermediate disintegration times.

Tablet hardness is obviously an important factor in determining the disintegration times (218) and dissolution rates. All tablet formulations were prepared so that they had an equal hardness of five units as measured by a Strong Cobb tablet hardness tester. Thus any differences in disintegration and dissolution characteristics were attributable solely to the differences in the diluents and granulating agents used.

During the tablet compression process, excessive capping of the tablets was found to be a problem. It was thought that this was due to the very 'fluffy' nature of the phentermine hydrochloride powder used in the formulations. This may have resulted in the trapping of air during the compression of the tablets. It was found that

this difficulty could be overcome by dissolving the drug in the granulating fluid prior to the granulation process. The phentermine powder was thus thoroughly wetted during the granulation procedure and satisfactory tablets were obtained after this minor manipulation.

A weight variation test was performed according to the procedure described in U.S.P. (208). A slight variation in the weights of the individual tablets was found. The greatest deviation found for a single tablet was +7.5% based on the mean of 20 tablets in formulation IV. The U.S.P. specifies that not more than two tablets should have a deviation of greater than 10% based on the mean of 20 tablets for a 250 mg tablet. The weight variations in all tablet formulations were found to be well within the specified range of the U.S.P.

From the results shown in Table III, it may be seen that all the four tablet formulations contained close to the expected amount of phentermine. A slight variation in drug content was found in each formulation (Table III). The greatest variation (less than 1%) in phentermine content was found in Formulation IV. This variation is within currently accepted limits, and may be attributed to the many steps involved in the manufacturing procedure for the tablets, particularly the granulation and compression processes.

The disintegration times of the four tablet

formulations were then examined using an official U.S.P. disintegration test. This was done for two reasons. Firstly, to determine whether the choice of disintegration medium had any marked effect on the disintegration times of the tablets, and secondly, it was hoped that there would be a correlation between disintegration times and dissolution rates in the various dissolution media examined. The results of the U.S.P. disintegration tests carried out are summarized in Table IV. The tests were performed using three disintegration media: distilled water, simulated gastric fluid, and simulated intestinal fluid. Reproducible disintegration times were obtained with each tablet formulation as is evident from Table IV. In the case where distilled water was the disintegration medium, it was found that the disintegration times of the tablet formulations were in the following order: II < III < I < IV. Thus formulation II disintegrated the fastest, and formulation IV the slowest. A similar order of disintegration was found in simulated gastric fluid. However, in the case of simulated intestinal fluid, a different order of disintegration was observed: II < I < III < IV. The disintegration times for formulation III and I were found to be reversed. The reason for this reversal is not known, and this was not studied further. However, it could be due to the different constituents present in simulated gastric and intestinal fluids; for example, differences in enzyme

constituents, or pH. This will be discussed later when the correlation of disintegration time with dissolution rate will be examined in more detail.

Before discussing the results obtained in the dissolution studies, a few remarks about the analytical procedure used are in order. Gas-liquid chromatographic analysis was used in this investigation to determine the amount of unchanged drug in urine. The method developed here had a high degree of accuracy, was relatively simple, and was specific for phentermine. OV-17 (3%) (stationary phase) on Chrom-W-AW-HMCS-HP (solid support) was found to be most satisfactory for the analysis. OV-11, OV-22 and OV-225 gave inferior results. Good symmetrical peaks were obtained for phentermine and the internal standard, chlorphentermine. The method required a short analysis time per sample (8.0 min) and gave good reproducibility during several months of continual use. The precision of the method was good as judged by a standard error of 0.643, based on ten determinations from the same solution containing 50 mcg of phentermine.

Although a number of methods are available to quantitate chromatographic peaks, the peak area ratios were used in this study to calculate unknown sample concentrations. Peak height ratios failed to give reproducible results. Chlorphentermine was used as an internal standard because it was also a basic drug, and therefore could be added to

the unknown urine sample before the extraction procedure. Further, chlorphentermine had a suitable retention time and was eluted as a symmetrical peak, and extracted quantitatively from urine samples.

A linear relationship was found to exist between the peak area ratio of phentermine and chlorphentermine over the anticipated concentration range of the drug excreted in the urine. It was also found that the re-establishment of a calibration curve at frequent intervals was unnecessary, since no differences were found when the calibration curve was repeated after one month. However, for the sake of reliability and an additional check, calibration curves were repeated at regular intervals.

The extraction procedure used in the present investigation was found to be very efficient, giving a recovery of 97 - 100% of the drug (Table V). The reproducibility of the extraction procedure was checked by extracting 12 urine samples, each containing 10 mcg phentermine; 10 mcg of the drug was recovered with a standard error of 0.15.

It was found in this study that the gas-liquid chromatographic procedure was an efficient method for quantitatively analyzing phentermine in either water or urine samples.

The analyses of the dissolution samples were performed using the GLC technique. Initially the same experimental technique was tried as for the urine samples,

but an interfering peak was found having the same retention time as the internal standard. This interfering peak was identified as the light mineral oil used as a lubricant in the manufacture of the tablets. This difficulty was overcome by a minor change in the extraction procedure. The samples were extracted with ether after acidifying them to pH <1.0 with 5N HCl. The light mineral oil was extracted out in the ether. The rest of the extraction procedure was the same as for the urine samples. This change in the extraction procedure necessitated the construction of a new calibration curve.

Two different GLC conditions were used for the analysis. All the dissolution results with distilled water as the dissolution media were obtained using Perkin-Elmer 990 Gas Chromatograph with isothermal operation at 150°. The remaining dissolution data were obtained with temperature programming between 130 - 160° using a Hewlett-Packard 5700A Gas Chromatograph. Both the methods gave good symmetrical peaks giving linear calibration curves in both cases.

Dissolution studies of all four tablet formulations of phentermine were performed in distilled water using the U.S.P. disintegration apparatus. This apparatus has been used by several workers to study the dissolution rates of drugs from the solid dosage forms (28). From examination of Figure 20, it is evident that this method was not

suitable for the study of comparative dissolution rates of phentermine from the tablets used in the present investigation. The dissolution rates were so close to each other that the small differences were virtually indistinguishable. The high degree of agitation produced by the apparatus was felt to be the probable cause of the small differences in dissolution rates observed. On the basis of these findings it may be suggested that the U.S.P. disintegration apparatus is not suitable for the dissolution studies from the fast-releasing solid dosage forms such as the tablet formulations in the present investigation. A similar suggestion has been made by Wagner (219).

Another possible method examined was the rotating basket method. The official U.S.P. dissolution apparatus (45) was used to study the dissolution of phentermine formulations in distilled water. An agitation intensity of 50 r.p.m. was used in this study instead of 150 r.p.m. as suggested in the U.S.P. XVIII for various tablets. An agitation intensity of 50 r.p.m. was recommended for the beaker method of Levy and Hayes (220) for products having small differences in <u>in vitro</u> drug release. Thus a reduced speed of agitation was selected in these studies.

The results obtained in this study suggested that this method was not suitable for the dissolution studies of the phentermine tablet formulations. As was the case with the U.S.P. disintegration apparatus, the four

dissolution curves (Figure 21) were too close to each other. Also, reproducibility and reliability were lacking from the results. This was thought to be due, firstly, to clogging of the basket by tablet adjuvants, particularly in the case of Formulation II in which calcium phosphate was the diluent, and Formulation IV in which dextrose was the diluent and acacia and starch were the granulating agents, and secondly, to improper mixing of the solution resulting in the collection of granules in the bottom of the dissolution vessel. These problems associated with the U.S.P. dissolution apparatus have been reported by other workers in the studies involving the dissolution of drugs from the solid dosage forms (46,47). Both clogging and lack of mixing have been blamed for the inconsistent results.

Another method which has given useful results in some studies is the rotating flask method. Using this method, Gibaldi and Weintraub (41) obtained a quantitative correlation between the in vitro dissolution rate and in vivo bioavailability of ASA tablets. The same method was used successfully in the present investigation, and good reproducible results were obtained. As the dissolution curves in Figure 22 indicate, a good spread in the dissolution rates was obtained, and it was possible to distinguish the four tablet formulations of phentermine on the basis of their different dissolution characteristics. The time required for 50% dissolution of the drug from each tablet

formulation was found to be statistically significantly different ($p > 0.005$) using the Student's t test.

The rotating flask apparatus seemed to meet the most important criteria for a suitable dissolution apparatus, namely good reproducibility in the results, operation under sink conditions, proper mixing of the contents, effective degree of agitation, reproducible stirring rate, and the ability to detect small differences in the dissolution rates. On the basis of these findings, further dissolution studies were conducted using the rotating flask method.

In order to obtain more meaningful information from the dissolution studies, dissolution experiments were carried out in simulated gastric and intestinal fluid using the rotating flask apparatus. It was thought that these studies would provide dissolution data having more physiological significance. Simulated gastric fluid (pH 1.2) containing pepsin, and simulated intestinal fluid (pH 7.5) containing pancreatin were used as the dissolution media. Several studies have been reported in the literature using above fluids as the dissolution media to determine the dissolution rates of drugs from solid dosage forms. Differences in dissolution rates, from solid dosage forms containing acidic and basic drugs, have been found, which were explained mainly by different pH effects on the solubility of the drugs (221). Other factors such as surface tension (222), viscosity (223), and presence of bile salts

(224) have been found to influence dissolution rates in some cases. From the results obtained for the dissolution of phentermine tablet formulations in distilled water (Tables XXI - XXIV) simulated gastric fluid (Tables XXV - XXVII), and simulated intestinal fluid (Tables XXIX - XXXII) the initial rates of dissolution were determined from the amounts of phentermine dissolved at different times. The initial dissolution rates are presented in Table XL.

Examination of Table XL revealed several interesting points. The most noteworthy was the fact that the dissolution medium had a pronounced effect on the dissolution rate of the drug from different individual formulations. In the three dissolution media used in these studies, the dissolution rates of the drug from different formulations followed the order II < I < III < IV, thus Formulation II exhibited the fastest release of the drug and Formulation IV the slowest. It will be recalled from the disintegration studies that a similar order of disintegration times was obtained in simulated intestinal fluid, but that the tablets disintegrated in a different order in distilled water and simulated gastric fluid. This phenomenon has been observed with other formulations in which no correlation was found between the disintegration times and dissolution rates (225). This confirms the fact that disintegration times are a poor and unreliable means of determining the dissolution rate.

Several factors may be responsible for observed

Table XL

The Initial Dissolution Rates[a] (mcg min^{-1})
of Phentermine Tablet Formulations in Various Dissolution Media

Formulation No.	Distilled Water	Simulated Gastric Fluid	Simulated Intestinal Fluid	Dilute Hydrochloric Acid	Phosphate Buffer
I	253.35	288.60	308.74		
II	324.35	321.71	367.04	316.81	380.32
III	183.75	258.74	226.99		
IV	153.60	208.29	95.70		

[a] mean of two determinations

differences in the dissolution rates of the drug in the three dissolution media. They are: pH effects, formulation factors, surface tension, viscosity, enzymes, and the wettability of the tablets. The effect of pH was thought to be the major factor responsible for the observed differences in the dissolution rates in different dissolution media since other studies (221) had shown this to have a marked effect. To study this in more detail, the pH of the dissolution media before and after the dissolution of drug from Formulation II was recorded (Table XXXV). It was found that there was a slight shift of +0.2 units in pH after the dissolution in the case of distilled water, and +0.15 units in the case of simulated intestinal fluid. Dissolution studies were also conducted in dilute hydrochloric acid solution (pH 1.2) and phosphate buffer (pH 7.5) since these solutions had the same pH as simulated gastric and intestinal fluids. No change was found in pH before and after the dissolution process. It was hoped that these studies would shed some light on the role of pH in the dissolution process. The initial dissolution rates of the drug from Formulation II under these conditions are presented in Table XL. It may be seen that the dissolution rates in simulated gastric fluid and dilute hydrochloric acid were very similar. A similar conclusion may be drawn from the dissolution rates observed in the case of simulated intestinal fluid and phosphate buffer. This suggests that

pH is one of the major factors controlling the observed differences in the dissolution rates of these phentermine tablet formulations. Another major factor appears to be formulation variables. Formulation factors such as different diluents and different granulating agents used in the preparation of tablets had a pronounced effect on the dissolution of phentermine in any of the three dissolution media. The use of starch and acacia as a granulating agent (Formulation IV) slowed down the dissolution rate of the drug in all dissolution media. This effect was very pronounced in the case of simulated intestinal fluid (Table XL). This was not further investigated.

Factors such as surface tension, viscosity and the presence of enzymes do not appear to make a major contribution to the dissolution of phentermine from the tablet dosage forms used in the present investigation. But further studies would be needed to confirm this.

Figures 24 and 27 show semi-logarithmic plots of percent drug undissolved versus time for each tablet formulation in simulated gastric and intestinal fluids, respectively. After an initial lag period, a straight line was obtained, which is indicative of first order release of phentermine from all the tablet formulations under study. To confirm this further, the percent dissolved values were plotted on a probability scale and time on a log scale according to the method of Wagner (97). The linear

relationships obtained (Figures 25 and 28) confirm the first order release of the drug from the formulations. This also supports the hypothesis that the surface area generated during the dissolution process follows a log-normal distribution (97).

It was felt that drug-diluent interaction could influence dissolution rates both in vitro and in vivo, since several examples of differences in bioavailabilities have been reported to be due to drug-excipient interaction. An excellent review in this area has been presented by Monkhouse and Lach (213). Thus binding studies between the drug and tablet diluents were conducted in order to eliminate the possibility that the differences in dissolution rates of phentermine tablet formulations under present investigation were due to this effect.

The equilibrium dialysis method of Patel and Foss (63) was used in the present investigation. It is known that the method gives best results when only one of the species transfers across the semipermeable membrane, and the other species does not cross the membrane. In the present case it was found that the membrane was permeable to both the drug and the diluents. To restrict the movement of the diluents across the membrane, an equal amount of the diluent was placed on both sides of the membrane. The results of the binding studies are presented in Table XXXVI. As can be seen from the figures presented in Table

XXXVI, an equal amount of phentermine was found on both sides of the membrane after equilibrium established at the end of 24 hours. This suggested that none of the tablet diluents, lactose, dextrose, or calcium phosphate, bind with phentermine in the presence of distilled water using this method.

Since calcium phosphate has a very low solubility in distilled water, a large excess of the solid was present on both sides of the membrane. This may have hindered in the transfer of the drug across the membrane. Therefore, the experiment was also carried out using a saturated solution of calcium phosphate. As is evident from Table XXXVI, no binding could be detected using this method.

From these results it may be concluded that drug-diluent interaction was not a reason for the observed differences in the dissolution rate of phentermine tablet formulations. Thus the differences observed were due solely to formulation factors and to different apparatuses and dissolution media used.

The urinary excretion studies were conducted in four normal human subjects who had no known history of any kidney or other related diseases. A cross-over design was used in this study. The results of the urinary excretion studies are presented in the Appendix. From examination of Figure 6 and Table A-21 in the Appendix, it is evident that when the urine pH was uncontrolled, the fluctuations in the amount of drug excreted in the urine were such that proper treatment

of the data became difficult if not impossible. Similar results were obtained by Beckett and Brooks (211) when they studied the urinary excretion of phentermine, and this phenomenon has also been observed with other basic drugs (149-150). Two factors were considered to be mainly responsible for such fluctuations in the urinary excretion rate of the basic drugs. These were urinary pH and urine flow rate. Fluctuations in the urinary pH cause variable amounts of the undissociated form of the drug to exist in the urine. The undissociated form of the drug should be readily reabsorbed from the kidney tubules. Thus any fluctuations in the urine pH should cause fluctuations in the amount of drug reabsorbed, and hence fluctuations in the urinary excretion rate. This should be particularly important in the case of phentermine which has a pK_a of 9.84 (211). Thus 99.99% would exist as the ionized form at pH 5.0 and 99% would exist as the ionized form at pH 7.0. For this reason, in the present investigation, the urine pH was controlled to the value of 5.0 $\pm$0.2 by administration of enteric-coated ammonium chloride tablets during the trial period, and the pH of each urine sample was carefully checked. Control of the urine pH eliminated or reduced the fluctuations in urinary excretion rate of phentermine as is evident from examination of Figure 7. Urine volume and urine flow rate have been reported to have some influence on the urinary excretion rate of drugs (150). It has been found

in this study that urine flow rate has some influence on the amount of phentermine excreted in this way. Two factors are known to control urine flow rate to a greater or lesser extent. One is the glomerular filtration rate (GFR) and the other the osmolality of the glomerular filtrate. Other factors are also involved. GFR is very difficult to control and it was not considered an important factor in this study. The osmolality of the glomerular filtrate is responsible to some extent for the amount of water reabsorption which takes place from the proximal tubules. Both these variables would tend to cause fluctuations in urine flow rate. In the present investigation no attempt was made to control the urine flow rate. It was hoped that because of the cross-over design of the study, problems associated with GFR and urine flow rate would be reduced to a minimum. The results obtained tended to support this assumption. In addition to this, circadian rhythms change the urine pH rhythmically at the same time of the day (226), and this may influence the excretion rate of a basic drug. However, this effect was eliminated since all the experiments were started at the same time of the day, and urine pH was controlled by the administration of ammonium chloride.

From Figure 8, it may be seen that when the pH of the urine was controlled, 75% of the unchanged drug was recovered in the urine compared to 42% over the same time period when the pH was not controlled. This was in good

agreement with the results of Beckett and Brooks (211). The higher recovery of the unchanged drug under controlled urine pH conditions was attributed by these workers to lower proportions of unionized phentermine at a lower urine pH, thereby minimizing the reabsorption of drug via the kidney tubules. However, metabolism of the drug may have a role in the reduction of these figures.

In these studies it has been assumed that the rate of metabolism was constant throughout the entire course of the study in a given individual. The kinetics of metabolism were not studied in this investigation, since none of the studies with other amphetamine-like drugs had shown any significant intra-subject variations in metabolite formation (211, 227). The possibility of inter-subject variation and its effect on the bioavailability was minimized by the cross-over design used in the urinary excretion studies.

The bioavailability of the phentermine tablet formulations were determined in comparison with a solution containing 10 mg of the drug. The cumulative amounts of unchanged drug excreted in the urine in 54 hours were used in the calculation of the bioavailability of each tablet formulation. In order to normalize the bioavailability values, the mean amounts of phentermine present in each tablet formulation (Table III) were used to calculate the percentage of the dose excreted unchanged in the urine. Normalization of the data was thought to be important in

eliminating problems due to the possible variations in the amount of the drug present in each tablet formulation.

A marked difference between the relative percent bioavailabilities of the four formulations was found. Examination of Table XXXVII indicated that Formulation II had the highest percent relative bioavailability followed by Formulations I, III and IV. It may be noted in Table XXXVII that in the case of Formulation II, two subjects (HC and GP) gave relative bioavailability values greater than 100%. The exact cause of this phenomenon is difficult to determine. It could be attributed to several factors, such as errors in the analytical procedure or collection of urine, or to physiological factors such as delayed or enhanced gastric emptying or changes in the metabolism of the drug.

The bioavailability data was subjected to analysis of variance using 4 X 4 latin square design. It was found that the differences in the bioavailabilities of different formulations were statistically significant.

In any study involving the bioavailability of a drug from a solid dosage form, an attempt may be made to correlate in vitro parameters, such as the disintegtation time or dissolution rate, with the bioavailability. A good and reliable correlation is useful for the prediction of the bioavailability of a given formulation from in vitro data. Prediction of bioavailability from an in vitro experiment

is very useful for quality control purposes, since the need for expensive and time-consuming bioavailability studies on a routine basis is eliminated. However, it should be kept in mind that the in vitro method is useful only for those formulations whose bioavailability has been determined and correlated with the in vitro data. Any changes in the formulation or the in vitro test conditions require the re-establishment of the in vitro - in vivo correlation.

In the present investigation, an attempt has been made to correlate the disintegration times or the dissolution rates obtained from the rotating flask method with the mean bioavailability. Several approaches have been examined in an attempt to reach meaningful conclusions. Many reports have appeared in the literature in which an attempt has been made to correlate the disintegration times of tablet formulations with their relative bioavailabilities. Usually such attempts were not successful because disintegration times did not provide an accurate measure of the drug actually released from a solid dosage form. However, an attempt was made in this study to correlate the mean disintegration times of these phentermine tablet formulations in three disintegration media: distilled water, simulated gastric fluid, and simulated intestinal fluid, with the mean bioavailabilities of the individual tablet formulations. A number of statistical approaches are available to examine the significance of such a correlation. In this study

Pearson's Product Moment Correlation, r (130) and the Wilcoxon Rank Order correlation (129) were applied. No statistically significant correlation was found between the disintegration times in distilled water ($r = 0.8156$), simulated gastric fluid ($r = 0.9017$) or simulated intestinal fluid ($r = 0.9125$) as was evident from the low values of Pearson's correlation coefficients obtained. None of these values are statistically significant. But it was found that statistically significant rank order correlation existed between the disintegration times in simulated intestinal fluid and mean bioavailability.

It is generally believed that the disintegration time of a tablet formulation is not a rate determining step in the disintegration-dissolution-absorption sequence of a drug. This phenomenon was found to be true with these phentermine tablet formulations since no correlation between the disintegration times and the bioavailability was found. To substantiate this, the correlation coefficients between the disintegration times and times required for 50% of the drug to dissolve in the three dissolution media were calculated, and no statistical correlation was found. Such results are not very surprising since it is a well established fact that the dissolution and not the disintegration of a tablet is the true measure of drug released in both *in vitro* and in *in vivo*. Though a rank order correlation was obtained between the disintegration times and

bioavailabilities, its use for the prediction of bioavailability from the disintegration time is questionable. It is because rank order correlation is, statistically speaking, less powerful than the Pearson's correlation approach.

Attempts were made to correlate the dissolution data with the in vivo results. Two approaches were used. In the first, the times required for 50% of the drug to dissolve (t_{50} diss.) for each formulation was correlated with the mean times for 50% of the drug to be absorbed (t_{50} abs.). In the second approach, the t_{50} diss. values were correlated with the mean bioavailabilities of each formulation. The t_{50} diss. values were obtained from Figures 22, 24 and 27, and t_{50} abs. values were taken from Tables VII - X. In both cases, t_{50} diss. values were also correlated with the t_{50} abs. and bioavailability values for each tablet formulation in each individual subject. The results are presented in Table XLI.

A highly statistically significant correlation was obtained between t_{50} dissolved and t_{50} absorbed values. Dissolution of the drug in each dissolution medium gave similar results. Using the pooled results from all four subjects, the following equations were obtained for simulated gastric fluid (Equation 17), simulated intestinal fluid (Equation 18), and distilled water (Equation 19).

Table XLI

Correlation Between t_{50} Dissolved and t_{50} Absorbed Values for Phentermine Tablet Formulations

Subject	Correlation Coefficients (r)[a]		
	Simulated Gastric Fluid	Simulated Intestinal Fluid	Distilled Water
All four Subjects	0.9957	0.9954	0.9995
JM	0.9966	0.9987	0.9938
HC	0.9946	0.9969	0.9972
SM	0.9933	0.9934	0.9995
GP	0.9917	0.9838[b]	0.9947

[a] all values are significant at p = 0.01 unless otherwise specified

[b] significant at p = 0.05

$$Y = 0.0344X + 2.2142 \quad \text{(Equation 17)}$$

$$Y = 0.0313X + 2.2387 \quad \text{(Equation 18)}$$

$$Y = 0.0350X + 2.2035 \quad \text{(Equation 19)}$$

where X represents the time required for 50% of the drug to dissolve in the dissolution medium, and Y is the mean time for 50% drug absorption obtained in a cross-over study.

These equations could be used to predict bioavailability of any of the formulations of phentermine considered in this investigation from the dissolution data. It also shows that there is a linear quantitative correlation between the dissolution rate and bioavailability.

A similar approach was tried to correlate t_{50} dissolved values and bioavailability data. The correlation coefficients are presented in Table XLII.

It may be seen that the values of r obtained in this case were lower than those obtained from t_{50} dissolved - t_{50} absorbed correlations. However, the correlations between t_{50} dissolved and bioavailabilities are still statistically significant, and could be used for predictive purposes. The following equations were derived which quantitatively relate the bioavailability data obtained in the cross-over study and the time required for 50% of the drug to dissolve in simulated gastric fluid (Equation 20), simulated intestinal fluid (Equation 21), and distilled

Table XLII

Correlation Between T_{50} Dissolved and Bioavailabilities for Phentermine Tablet Formulations

Subject	Correlation Coefficient[a]		
	Simulated Gastric Fluid	Simulated Intestinal Fluid	Distilled Water
All four Subjects	-0.9732	-0.9691	-0.9920[b]
JM	-0.9755	-0.9756	-0.9933[b]
HC	-0.9690	-0.9641	-0.9894
SM	-0.9455	-0.9307[c]	-0.9592
GP	-0.9523	-0.9586	-0.9763

[a] all values are significant at p = 0.05 unless otherwise specified

[b] significant at p = 0.01

[c] not significant

water (Equation 22).

$$Y = -1.2319X + 117.1720 \qquad \text{(Equation 20)}$$

$$Y = -1.1144X + 116.1800 \qquad \text{(Equation 21)}$$

$$Y = -1.2726X + 118.0240 \qquad \text{(Equation 22)}$$

where X is the time required for 50% of the drug to dissolve in the in vitro experiment, and Y is the percent relative bioavailability obtained in a cross-over study.

Either of the two approaches could be utilized to predict the bioavailability of a phentermine tablet formulation from the in vitro dissolution data obtained using the rotating flask method under the conditions utilized in the present investigation. Although the level of significance was lower in the case of t_{50} diss. - bioavailability correlations, this method would be satisfactory for predictive purposes since the 100 r^2 value was always higher than 90. It was suggested by Wagner (228) that the 100 r^2 value should be higher than 90 for predictive purposes. Also, the bioavailability data was obtained with relative ease by comparing the cumulative amounts of unchanged drug excreted in the urine for each tablet formulation. Knowledge of pharmacokinetic behaviour of phentermine was not absolutely essential to get the bioavailability which was based on the amount of the unchanged drug excreted in the urine, whereas it was essential in construction of percent

absorbed-time plots by the Wagner-Nelson method.

It may be concluded from these observations that disintegration times are not a reliable *in vitro* parameter for predicting the bioavailability of phentermine from the tablets studied in this investigation. The dissolution rates in simulated gastric fluid, simulated intestinal fluid and distilled water using the rotating flask apparatus gave a reliable quantitative correlation with the bioavailability data. It may also be concluded on the basis of the correlation obtained that the dissolution step is the rate-determining step in absorption of phentermine after oral administration of the drug. This is based on the fact that the drug must first dissolve before it is absorbed from the gastrointestinal tract. Thus the rate of dissolution of the drug will affect the amount of the drug which is absorbed in a given time, hence its bioavailability. A dissolution controlled absorption phenomenon has been observed in the case of many drugs. It was also found that the differences observed in the initial dissolution rates in the three dissolution media did not significantly alter the *in vitro* - *in vivo* correlations. On this basis, any of the dissolution media, simulated gastric fluid, simulated intestinal fluid, or distilled water, could be used in the dissolution studies using rotating flask apparatus under the conditions specified in this investigation.

Before closing this discussion, some mention must

be made of the pharmacokinetic parameters that were obtained from the urinary excretion data. Pharmacokinetic parameters such as absorption and excretion rate constants for any drug are very useful in determining the fate of a drug in the body. The kinetic behaviour of phentermine after oral administration of the drug in solution or tablet form was studied using both analog and digital computers. It must be kept in mind that determination of rate constants obtained by the urinary excretion data are questionable particularly with regard to the absorption rate constant (K_a). The excretion rate constant (K_u) obtained from the excretion data should be reasonably accurate. It is well known that the blood data are needed for reliable determination of rate constants. In the present investigation, an attempt was made to obtain blood data in dogs; however, it was unsuccessful because of the low concentrations of the drug present in the blood following a 10 mg dose of phentermine after I.V. administration. Higher doses of the drug could not be given to the dogs since phentermine exerted adverse effects on the blood pressure and heart, as shown by electrocardiograms obtained during the study.

A one compartmental model, shown in Figure 1, was assumed to apply to phentermine following oral administration of the drug. A good fit between the observed cumulative amounts of phentermine excreted in the urine and the

computer predicted curves was obtained in each case. The rate constants obtained from the analog computer were used as the initial estimates needed for the simulation of the model by the digital computer. To obtain a good fit, it was necessary to subtract lag times obtained from the analog computer from the urine collection times. This modified time scale was used in fitting the data using the digital computer.

There was a fair agreement between the excretion rate constants obtained from the analog and digital computers. But there was a great variability in the absorption rate constant obtained using each method. This rate constant is not a true absorption rate constant, but is made up of dissolution and absorption rate constants in the case of the tablet formulations. The K_a obtained following the administration of the solution is a true measure of the absorption rate constant in each subject. On the basis of good fit obtained, the validity of the assumption that phentermine follows one compartmental model in man could be claimed to be confirmed. In an attempt to improve the fit, a two compartmental model was also tried. It was found that it was very difficult to fit the data using the analog computer due to limitations in selecting very small rate constants. However, the rate constants obtained from the closest fit from the analog computer were supplied to the digital computer program as initial estimates. The fit

obtained was not as good as for the system using one compartmental model. Normally an improvement would have been obtained, but it seems that the rate constants supplied were not correct. Since a good fit was obtained between the experimental points and computer generated curves using one compartmental model, further work using the two compartmental model was discontinued.

The biological half-life of phentermine in man was found to be 7 - 12 hours. Some intra- and inter-subject variations were found among the four individuals used in this study. The variations in K_a values are higher than those in K_u values. Intra- and inter-subject variations have been found to occur with many drugs. These variations probably result from variations in the subjects' urinary flow rates, urine pH's, and kidney functions.

SUMMARY

The main conclusions which may be drawn from the work described in this thesis are summarized in the following paragraphs.

A sensitive and reliable procedure for quantitative analysis of phentermine was developed using gas-liquid chromatographic procedure.

It was found possible to prepare four tablet formulations of phentermine by controlling formulation variables so that the disintegration and dissolution characteristics of the tablet formulations were different. This was achieved by changing the diluents and granulating fluids used in each formulation.

The dissolution rates were determined in various dissolution media. It was found that the pH of the dissolution medium had some influence on the initial dissolution rates of phentermine from various phentermine tablet formulations.

The bioavailabilities of the four tablet formulations in man were determined on the basis of urinary excretion of the drug under controlled urinary pH conditions. These studies indicated that by changing formulation variables it was possible to obtain tablets of phentermine having significantly different bioavailabilities.

A quantitative correlation was found between the dissolution rates and bioavailability of phentermine tablet formulations. These correlations may be utilized in

predicting the bioavailability of phentermine from the dissolution data. A similar prediction cannot be made from the disintegration times since no correlation between the disintegration time and bioavailability was found.

The pharmacokinetic behaviour of phentermine in man was examined, using analog and digital computers, from the urinary excretion data after oral administration of solution and tablets. Some of the pharmacokinetic parameters which determine the fate of phentermine in the body were determined.

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APPENDIX

Table A-1

Subject: JM

Urine pH: Acid

Dose: Phentermine Hydrochloride Solution
(10 mg phentermine)

Time (hr)	Urine Flow Rate ($ml\ min^{-1}$)	Urine pH	Excretion Rate ($mcg\ min^{-1}$)	Phentermine Excreted (mcg)
1	3.25	4.91	0.95	57
2	1.24	5.02	2.55	210
3	1.58	5.11	3.62	427
4	0.98	4.98	9.62	1004
5	0.91	5.08	9.98	1603
6	1.00	5.20	8.10	2089
10	1.39	5.00	6.62	3678
14	1.50	5.00	5.28	4945
16	2.16	4.95	3.53	5369
24	1.15	5.15	2.29	6468
30	0.99	-	1.55	7026
36	2.11	5.19	0.61	7246
40	1.36	5.08	0.38	7337
48	0.96	4.95	0.23	7447
54	0.76	5.11	0.16	7505

Table A-2

Subject: JM

Urine pH: Acid

Dose: One Tablet of Formulation I

Time (hr)	Urine Flow Rate ($ml\ min^{-1}$)	Urine pH	Excretion Rate ($mcg\ min^{-1}$)	Phentermine Excreted (mcg)	% Absorbed
1	2.45	5.00			
2	1.36	5.00	1.50	90	10
3	1.48	5.17	2.16	220	46
4	1.99	5.11	2.20	352	74
5	3.45	4.95	4.02	593	84
6	1.24	5.21	4.98	892	94
10	0.98	-	7.21	2622	
14	0.94	-	5.00	3822	
16	1.09	5.21	4.36	4345	
24	1.00	4.99	2.29	5444	
30	2.35	5.17	1.15	5858	
36	4.45	5.16	0.68	6103	
40	1.94	5.00	0.45	6211	
48	0.96	4.99	0.20	6307	
54	1.59	5.20	0.13	6354	

Table A-3

Subject: JM

Urine pH: Acid

Dose: One Tablet of Formulation II

Time (hr)	Urine Flow Rate ($ml\ min^{-1}$)	Urine pH	Excretion Rate ($mcg\ min^{-1}$)	Phentermine Excreted (mcg)	% Absorbed
1	1.16	5.09			
2	2.34	5.21	0.80	48	7
3	2.16	5.08	2.53	200	67
4	1.91	5.11	3.13	388	81
5	0.98	5.22	4.33	648	93
6	0.91	-	6.92	1063	
10	0.72	4.96	9.02	3227	
14	0.88	4.98	5.18	4470	
16	1.94	5.16	4.62	5024	
24	2.16	-	2.70	6320	
30	1.00	5.12	1.33	6799	
36	4.36	5.00	0.80	7087	
40	4.55	5.00	0.48	7202	
48	1.65	5.29	0.27	7332	
54	1.32	5.15	0.11	7372	

Table A-4

Subject: JM

Urine pH: Acid

Dose: One Tablet of Formulation III

Time (hr)	Urine Flow Rate ($ml\ min^{-1}$)	Urine pH	Excretion Rate ($mcg\ min^{-1}$)	Phentermine Excreted (mcg)	% Absorbed
1	1.46	4.90			
2	2.00	5.16	0.80	48	7
3	1.36	5.11	1.45	135	37
4	1.74	5.10	1.65	234	65
5	0.92	5.17	2.75	399	81
6	1.00	5.00	3.15	588	86
10	0.72	4.98	7.10	2292	
14	2.32	5.21	4.68	3415	
16	1.94	-	3.55	3841	
24	1.64	5.28	2.05	4825	
30	4.65	4.99	1.15	5239	
36	3.20	5.15	0.59	5451	
40	1.88	5.16	0.45	5559	
48	1.03	5.08	0.21	5660	
54	1.93	5.20	0.11	5700	

Table A-5

Subject: JM

Urine pH: Acid

Dose: One Tablet of Formulation IV

Time (hr)	Urine Flow Rate ($ml\ min^{-1}$)	Urine pH	Excretion Rate ($mcg\ min^{-1}$)	Phentermine Excreted (mcg)	% Absorbed
1	1.64	5.00			
2	3.22	5.01	1.35	81	10
3	0.64	5.13	1.75	186	40
4	0.85	5.11	1.80	294	54
5	1.94	4.96	2.70	457	72
6	1.00	5.20	3.75	682	82
10	1.32	-	6.00	2122	
14	1.39	-	4.56	3216	
16	1.78	5.00	3.19	3599	
24	1.02	4.91	1.85	4487	
30	0.85	5.11	0.88	4804	
36	0.96	5.35	0.52	4991	
40	2.29	5.14	0.33	5070	
48	1.07	5.08	0.17	5152	
54	1.82	5.26	0.13	5199	

Table A-6

Subject: HC

Urine pH: Acid

Dose: Phentermine Hydrochloride Solution
(10 mg phentermine)

Time (hr)	Urine Flow Rate ($ml\ min^{-1}$)	Urine pH	Excretion Rate ($mcg\ min^{-1}$)	Phentermine Excreted (mcg)
1	1.36	5.01	1.20	72
2	1.55	5.16	1.74	176
3	1.09	5.11	3.51	387
4	0.85	5.03	8.42	892
5	0.96	4.95	8.30	1390
6	0.99	5.22	6.82	1799
10	1.08	-	5.46	3109
14	1.55	5.27	5.11	4336
16	1.49	5.01	3.15	4714
24	1.75	5.11	2.40	5866
30	1.85	4.95	1.31	6337
36	0.82	4.99	0.82	6534
40	1.72	5.17	0.58	6673
48	1.69	5.36	0.42	6875
54	2.25	5.14	0.18	6940
60	3.71	5.11	0.16	6998

Table A-7

Subject: HC

Urine pH: Acid

Dose: One Tablet of Formulation I

Time (hr)	Urine Flow Rate ($ml\ min^{-1}$)	Urine pH	Excretion Rate ($mcg\ min^{-1}$)	Phentermine Excreted (mcg)	% Absorbed
1	1.18	5.15	0.65	39	7
2	3.25	5.29	0.78	85	9
3	1.99	5.01	2.32	225	53
4	1.85	4.96	4.02	466	70
5	1.16	5.11	4.31	724	85
6	1.75	5.18	5.72	2097	94
10	0.95	5.02	3.81	3012	
14	0.75	5.11	4.00	3492	
16	2.14	5.14	2.21	4553	
24	1.53	5.35	1.52	5100	
30	1.39	5.00	0.86	5409	
36	1.44	5.13	0.62	5558	
40	1.46	4.98	0.36	5731	
48	1.00	5.19	0.26	5824	
54	2.02	5.06	0.14	5875	
60	0.95	5.13	0.12	5918	

Table A-8

Subject: HC

Urine pH: Acid

Dose: One Tablet of Formulation II

Time (hr)	Urine Flow Rate ($ml\ min^{-1}$)	Urine pH	Excretion Rate ($mcg\ min^{-1}$)	Phentermine Excreted (mcg)	% Absorbed
1	2.00	5.11			
2	1.54	5.08	1.22	73	10
3	1.66	4.95	2.94	249	58
4	1.35	5.00	3.48	457	86
5	1.99	5.16	6.60	853	92
6	3.25	5.22	8.25	1348	
10	2.55	5.11	6.15	2824	
14	6.35	4.96	6.22	4316	
16	0.99	4.99	5.01	4917	
24	1.54	5.21	2.82	6270	
30	1.34	5.17	1.46	6796	
36	1.30	5.18	1.25	7246	
40	1.08	5.00	0.74	7423	
48	2.15	5.09	0.39	7610	
54	1.35	5.19	0.32	7725	
60	1.11	-	0.17	7786	

Table A-9

Subject: HC

Urine pH: Acid

Dose: One Tablet of Formulation III

Time (hr)	Urine Flow Rate ($ml\ min^{-1}$)	Urine pH	Excretion Rate ($mcg\ min^{-1}$)	Phentermine Excreted (mcg)	% Absorbed
1	4.15	5.04			
2	1.99	5.11	0.52	31	7
3	2.15	5.13	0.61	68	37
4	1.06	5.20	1.42	153	65
5	1.36	4.95	1.90	267	80
6	1.11	4.99	2.46	415	89
10	0.84	5.14	4.68	1038	
14	0.99	5.16	4.31	2572	
16	1.87	5.11	2.72	2899	
24	1.95	5.23	2.23	3969	
30	2.32	5.11	1.11	4368	
36	1.16	5.00	0.84	4671	
40	1.17	5.10	0.49	4788	
48	1.11	5.19	0.33	4947	
54	1.35	5.11	0.18	5012	
60	1.55	5.20	0.13	5059	

Table A-10

Subject: HC

Urine pH: Acid

Dose: One Tablet of Formulation IV

Time (hr)	Urine Flow Rate ($ml\ min^{-1}$)	Urine pH	Excretion Rate ($mcg\ min^{-1}$)	Phentermine Excreted (mcg)	% Absorbed
1	1.95	5.16			
2	1.96	5.18	0.45	27	4
3	1.09	5.03	0.89	80	35
4	1.45	5.04	0.95	137	61
5	0.57	5.01	1.90	251	76
6	1.36	4.99	2.22	384	82
10	1.88	5.11	3.20	1152	
14	1.36	5.13	3.95	2100	
16	5.34	-	3.05	2466	
24	2.11	-	1.52	3195	
30	0.91	5.07	1.44	3713	
36	2.15	5.15	0.76	3987	
40	1.16	5.14	0.51	4105	
48	1.09	5.25	0.32	4263	
54	5.82	5.09	0.23	4346	
60	2.65	5.04	0.14	4396	

Table A-11

Subject: SM

Urine pH: Acid

Dose: Phentermine Hydrochloride Solution
(10 mg phentermine)

Time (hr)	Urine Flow Rate ($ml\ min^{-1}$)	Urine pH	Excretion Rate ($mcg\ min^{-1}$)	Phentermine Excreted (mcg)
1	1.46	5.11	0.8	48
2	1.40	5.00	2.15	177
3	2.00	5.10	6.37	559
4	1.55	4.95	5.55	892
5	3.57	5.18	5.10	1198
6	0.85	5.15	4.73	1482
10	4.36	5.01	3.50	2322
14	1.00	5.06	2.96	3032
16	1.72	5.20	2.56	3339
24	1.35	5.20	1.51	4064
30	1.85	-	0.91	4392
36	1.36	5.14	0.55	4590
40	2.05	5.09	0.46	4700
48	1.03	5.08	0.22	4806
54	3.32	-	0.15	4860

Table A-12

Subject: SM

Urine pH: Acid

Dose: One Tablet of Formulation I

Time (hr)	Urine Flow Rate ($ml\ min^{-1}$)	Urine pH	Excretion Rate ($mcg\ min^{-1}$)	Phentermine Excreted (mcg)	% Absorbed
1	1.88	5.13			
2	2.15	5.11	1.00	60	7
3	1.15	5.10	1.56	154	52
4	1.00	5.10	2.29	291	70
5	1.96	5.10	4.67	571	85
6	1.72	5.00	4.25	826	92
10	1.35	5.15	3.75	1726	
14	4.36	5.16	2.62	2355	
16	1.44	5.11	2.30	2631	
24	2.15	5.03	1.41	3341	
30	1.62	4.96	0.85	3647	
36	1.38	4.99	0.53	3774	
40	1.55	5.11	0.41	3872	
48	1.59	5.23	0.23	3982	
54	1.11	5.18	0.14	4042	

Table A-13

Subject: SM

Urine pH: Acid

Dose: One Tablet of Formulation II

Time (hr)	Urine Flow Rate ($ml\ min^{-1}$)	Urine pH	Excretion Rate ($mcg\ min^{-1}$)	Phentermine Excreted (mcg)	% Absorbed
1	1.38	5.00			
2	1.52	5.15	0.82	49	6
3	2.05	5.11	1.95	166	59
4	2.96	4.96	3.25	361	85
5	1.51	4.99	5.59	695	94
6	1.03	5.11	5.07	1000	
10	1.00	5.23	4.41	2058	
14	0.85	5.31	3.11	2804	
16	1.08	5.10	2.50	3104	
24	1.52	5.11	1.65	3896	
30	1.39	5.00	0.90	4220	
36	1.49	5.13	0.64	4450	
40	0.99	5.15	0.45	4612	
48	1.11	-	0.15	4684	
54	2.53	-	0.16	4742	

Table A-14

Subject: SM

Urine pH: Acid

Dose: One Tablet of Formulation III

Time (hr)	Urine Flow Rate ($ml\ min^{-1}$)	Urine pH	Excretion Rate ($mcg\ min^{-1}$)	Phentermine Excreted (mcg)	% Absorbed
1	1.11	5.18			
2	1.45	5.19	0.52	31	5
3	2.36	5.00	0.80	79	35
4	1.85	5.00	1.05	142	65
5	1.11	5.09	1.51	233	74
6	3.25	5.13	2.45	380	87
10	1.73	5.18	4.27	1405	
14	1.99	4.99	3.00	2125	
16	2.08	-	2.06	2372	
24	1.36	5.20	1.86	3265	
30	1.11	5.11	0.91	3593	
36	1.37	5.16	0.57	3798	
40	1.41	5.00	0.41	3896	
48	1.30	5.22	0.25	4016	
54	1.35	5.09	0.15	4070	

Table A-15

Subject: SM

Urine pH: Acid

Dose: One Tablet of Formulation IV

Time (hr)	Urine Flow Rate ($ml\ min^{-1}$)	Urine pH	Excretion Rate ($mcg\ min^{-1}$)	Phentermine Excreted (mcg)	% Absorbed
1	1.40	5.95			
2	1.58	5.22	0.49	29	4
33	1.69	5.11	0.57	63	33
4	2.36	5.00	0.91	118	57
5	1.66	5.20	1.03	268	65
6	1.00	5.05	1.46	1101	80
10	1.39	5.18	3.47	1874	
14	2.36	4.95	3.22	2184	
16	5.32	4.99	2.58	2870	
24	1.06	5.21	1.43	3212	
30	1.56	5.19	0.95	3435	
36	0.99	5.11	0.62	3548	
40	1.25	5.00	0.47	3678	
48	1.49	5.11	0.27	3736	
54	1.98	5.13	0.16		

Table A-16

Subject: GP

Urine pH: Acid

Dose: Phentermine Hydrochloride Solution
(10 mg phentermine)

Time (hr)	Urine Flow Rate ($ml\ min^{-1}$)	Urine pH	Excretion Rate ($mcg\ min^{-1}$)	Phentermine Excreted (mcg)
1	3.11	5.10		
2	3.05	5.10	1.21	73
3	4.62	5.10	1.65	172
4	1.96	5.16	2.09	297
5	1.84	5.11	2.66	457
6	1.11	4.96	3.28	654
10	1.96	5.12	5.99	2092
14	2.08	5.20	5.22	3345
16	1.55	5.16	3.84	3806
24	1.16	5.09	3.21	5346
30	1.36	5.11	1.91	6033
36	1.09	-	1.35	6519
40	1.68	5.19	0.86	6725
48	1.45	5.16	0.63	7027
54	2.19	5.12	0.25	7117
60	1.67	-	0.29	7221

Table A-17

Subject: GP

Urine pH: Acid

Dose: One Tablet of Formulation I

Time (hr)	Urine Flow Rate ($ml\ min^{-1}$)	Urine pH	Excretion Rate ($mcg\ min^{-1}$)	Phentermine Excretion (mcg)	% Absorbed
1	2.46	5.00			
2	1.88	5.15	2.02	121	9
3	0.99	5.11	3.61	338	66
4	1.01	5.10	6.00	698	80
5	1.11	4.99	8.22	1071	96
6	0.96	4.98	7.16	1501	
10	0.85	5.12	5.82	2898	
14	0.61	5.20	4.90	4074	
16	0.72	5.09	3.35	4464	
24	0.85	5.18	2.60	5712	
30	0.98	5.11	1.76	6346	
36	1.00	5.00	1.03	6717	
40	0.99	5.16	0.81	6911	
48	0.96	5.17	0.46	7132	
54	1.20	5.10	0.39	7272	
60	1.55	5.10	0.19	7340	

Table A-18

Subject: GP

Urine pH: Acid

Dose: One Tablet of Formulation II

Time (hr)	Urine Flow Rate ($ml\ min^{-1}$)	Urine pH	Excretion Rate ($mcg\ min^{-1}$)	Phentermine Excretion (mcg)	% Absorbed
1	3.72	5.00			
2	2.61	5.00	1.62	97	10
3	2.00	-	2.22	230	78
4	1.21	5.16	4.86	522	88
5	0.98	4.95	5.10	852	91
6	0.96	5.11	7.32	1291	
10	0.90	5.16	6.22	2784	
14	0.99	5.09	5.40	4080	
16	1.00	5.01	3.61	4513	
24	0.63	5.20	2.90	5905	
30	0.54	5.10	1.65	6499	
36	0.87	5.07	1.25	6949	
40	0.96	-	0.87	7158	
48	0.98	5.16	0.56	7427	
54	1.20	5.20	0.42	7578	
60	0.84	-	0.22	7657	

Table A-19

Subject: GP

Urine pH: Acid

Dose: One Tablet of Formulation III

Time (hr)	Urine Flow Rate ($ml\ min^{-1}$)	Urine pH	Excretion Rate ($mcg\ min^{-1}$)	Phentermine Excretion (mcg)	% Absorbed
1	2.51	5.10			
2	3.66	5.11	1.41	85	5
3	1.43	5.06	1.36	167	57
4	1.22	5.21	1.97	285	74
5	1.00	5.06	2.80	453	87
6	0.96	5.11	4.90	747	93
10	0.99	4.95	5.91	2165	
14	0.98	4.99	4.51	3247	
16	1.36	5.00	3.48	3665	
24	2.11	5.25	2.71	4966	
30	1.87	5.17	1.51	5510	
36	1.34	5.11	1.27	5967	
40	2.65	5.12	0.71	6137	
48	0.99	5.18	0.42	6339	
54	0.91	-	0.34	6461	
60	0.82	5.21	0.20	6533	

Table A-20

Subject: GP

Urine pH: Acid

Dose: One Tablet of Formulation IV

Time (hr)	Urine Flow Rate ($ml\ min^{-1}$)	Urine pH	Excretion Rate ($mcg\ min^{-1}$)	Phentermine Excretion (mcg)	% Absorbed
1	3.46	5.16	1.52	91	
2	2.11	5.11	7.62	548	4
3	0.99	5.09	7.74	1012	43
4	1.52	5.02	6.00	1372	70
5	0.84	5.11	5.40	1696	80
6	0.95	5.16	5.61	2062	92
10	1.00	5.26	5.01	3264	
14	0.84	5.00	3.42	3885	
16	0.65	5.16	2.70	4209	
24	0.84	5.15	1.93	5135	
30	0.61	5.00	1.20	5567	
36	0.72	4.96	0.82	5862	
40	0.98	5.11	0.63	6013	
48	0.99	-	0.39	6200	
54	1.52	5.19	0.28	6301	
60	0.95	5.17	0.17	6362	

Table A-21

Subject: JM

Urine pH: Uncontrolled

Dose: Phentermine Hydrochloride Solution
(10 mg phentermine)

Time (hr)	Urine Flow Rate ($ml\ min^{-1}$)	Urine pH	Excretion Rate ($mcg\ min^{-1}$)	Phentermine Excreted (mcg)
1	1.51	5.80	1.33	80
2	0.70	6.35	2.26	215
3	0.88	6.70	1.73	319
4	0.57	6.65	2.25	455
5	1.91	6.15	2.59	610
7	3.87	5.65	4.36	1004
11	0.97	5.80	2.44	1590
18	0.82	5.85	3.02	3005
22	0.93	5.45	1.08	3264
24	0.34	5.95	0.43	3329
28	1.81	6.00	0.27	3382
30	0.85	6.25	0.71	3447
34	1.42	6.50	1.13	3780
36	1.30	5.75	0.47	3858
47	1.55	6.60	0.48	4132
55	1.50	6.45	0.46	4295